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(54) Title: CATERPILLER GENE FAMILY

(57) Abstract: The present invention relates to a new family of structurally and functionally related nucleic acids and proteins, designed the CATERPILLER family, which is characterized by landmark structural motifs including a nucleotide binding domain and leucine-rich repeat domains.

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CATERPILLER Gene Family

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RELATED APPLICATION INFORMATION

This application claims the benefit of United States Provisional Application No. 60/376,626; filed April 30, 2002, which is incorporated by reference herein in its entirety.

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STATEMENT OF FEDERAL SUPPORT

This invention was made, in part, with government support under grant numbers AI29564, AI45580, AI41751, DK38108, T32 CA09156 from the National Institutes of Health. The United States government has certain rights to this invention.

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FIELD OF THE INVENTION

The present invention relates to a new family of genes termed CATERPILLER which are characterized by the presence of landmark motifs including nucleotide binding domain (NBD) and leucine rich repeat (LRR) domains.

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BACKGROUND OF THE INVENTION

A number of genes with nucleotide-binding domain (NBD) and leucine rich repeat (LRR) domains are rapidly emerging as important in apoptosis, immune and inflammatory disorders. These include CIITA, Nod1/CARD4, Nod2/CARD15, DEFCAP/CARD7/NALP1 and CIAS1/PYPAF1. CIITA, Nod2, and CIAS1 are linked to a number of immunologic disorders. CIITA is the master transcriptional regulator of class II MHC (MHCII). Genetic lesions in CIITA cause an immunodeficiency, Type II Bare Lymphocyte Syndrome (BLS) (Group A) (Steimle et al., (1993) *Cell* 75:135). Recently, mutations in Nod2 and CIAS1 have been linked to four immunologic and inflammatory disorders (Ogura et al., (2001) *Nature* 411:603; Hugot et al., (2001) *Nature* 411:599;

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Hoffman et al., (2001) *Nat. Genet.* **29**:301; Manji et al., (2002) *J. Biol. Chem.* **277**:11570).

CIITA was isolated using a complementation cloning strategy to restore MHC II expression to a MHC II deficient cell line (Steimle et al., (1993) *Cell* **75**:135). CIITA is a master regulator of transcription, responsible for both interferon- γ and constitutive expression of MHC II and related genes (Harton et al., (2000) *Mol. Cell. Biol.* **20**:6185; Reith et al., (2001) *Annu. Rev. Immunol.* **19**:331). The N-terminal activation domain of CIITA is necessary for transcriptional activation (Harton et al., (2000) *Mol. Cell. Biol.* **20**:6185). The centrally located NBD of CIITA contains a GTP-binding domain required for nuclear import (Harton et al., (2000) *Mol. Cell. Biol.* **20**:6185). CIITA undergoes self-association involving sequences in its NBD, C-terminal LRRs, and N-terminus (Ting et al., (2002) *Cell* **109** (Suppl.): S21).

When CIITA was first discovered, initial searches for CIITA-related genes produced no significant matches. Nod1, an activator of caspase-9-mediated apoptosis and NF- κ B, also having an NBD and C-terminal LRRs was the first described protein similar to CIITA in domain organization (Bertin et al., (1999) *J. Biol. Chem.* **274**:12955; Iohara et al., (1999) *J. Biol. Chem.* **274**:14560. Nod2, with functions similar to Nod1, has been strongly implicated in Crohn's disease (Ogura et al., (2001) *Nature* **411**:603; Hugot et al., (2001) *Nature* **411**:599; Ogura et al., (2001) *J. Biol. Chem.* **276**:4812), and in familial granulomatous synovitis (Blau syndrome) (Miceli-Richard et al., (2001) *Nat. Genet.* **29**:19). Most recently, patients with familial cold autoinflammatory syndrome (familial cold urticaria) and Muckle-Wells syndrome were found to have mutations in a new gene called CIAS1, which has a pyrin domain, NBD and LRR (Hoffman et al., (2001) *Nat. Genet.* **29**:301). These syndromes are associated with a CIAS1 splice variant called cryopyrin. These proteins may be similar to plant disease resistance proteins (R proteins) which detect pathogens and initiate defense mechanisms including MAP kinase activation, oxygen radical formation, salicylate production, induced transcription of kinases and transcription factors, and rapid cell death (Dangl et al., (2001) *Nature* **411**:826). Many of these plant

proteins have an NBD and LRRs and may represent the oldest examples of proteins using this CIITA-like domain arrangement.

SUMMARY OF THE INVENTION

5 The present invention is based, in part, on the discovery of a new family of genes that the inventors have designated the CATERPILLER (CARD, Transcription Enhancer, R(purine)-binding, Pyrin, Lots of Leucine Repeats) gene family. Some members of this family were previously known, but were not recognized as belonging to a large family of structurally and
10 functionally related molecules. The advent of the nearly complete human genome sequence facilitated a search for sequences related to these proteins. The inventors describe the identification of additional CATERPILLER genes encoding mammalian NBD/LRR proteins. This analysis predicts at least twenty-two CATERPILLER genes in the human
15 genome, many of which occur in clusters on individual chromosomes.

 The CATERPILLER genes are implicated in inflammatory states, apoptosis, sepsis and infection among other conditions and provide an important new class of therapeutic targets.

 Accordingly, as one aspect, the invention provides an isolated nucleic
20 acid encoding a polypeptide selected from the group consisting of: (a) a Monarch-1 polypeptide; (b) a CATERPILLER 11.2 polypeptide; (c) a CATERPILLER 11.3 polypeptide; (d) a CATERPILLER 16.1 polypeptide; (e) a CATERPILLER 16.2 polypeptide; and (f) a functional fragment of any of (a) to (e). Also provided are isolated nucleic acids encoding a functional fragment
25 of CIAS1.

 As a further aspect, the invention provides polypeptides encoded by the isolated nucleic acid sequences. Further provided are cells comprising the isolated nucleic acids and polypeptides of the invention.

 As yet another aspect, the invention provides an antibody that
30 specifically binds to the polypeptides of the invention.

 As still another aspect, the invention provides a method of modulating the cellular activity of a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3,

CATERPILLER 16.1, CATERPILLER 16.2, and a functional fragment thereof, comprising introducing into a cell a compound that modulates the activity of the polypeptide in an amount effective to modulate the activity of the polypeptide in the cell. Also provided are methods of modulating cellular inflammatory responses, apoptosis, and a Toll-like receptor pathway activity. In particular embodiments, the compound is an isolated nucleic acid encoding a polypeptide of the invention, an antisense oligonucleotide, an siRNA, or an antibody. In other representative embodiments, the compound is an antisense oligonucleotide or siRNA that is targeted against the CATERPILLER nucleic acid. In other embodiments, the compound is an antibody that binds to the CATERPILLER polypeptide. The methods can be carried out in cultured cells or *in vivo*.

The present invention further provides screening methods using the nucleic acids and polypeptides of the invention as targets. The screening methods can be carried out in cell-free assays, in cultured cells or in live organisms, such as transgenic non-human animals, plants, fungi or bacteria.

As one particular aspect, the invention provides a method for identifying a compound that binds to a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2, and a functional fragment of any of the foregoing, comprising: contacting the polypeptide with a test compound under conditions whereby binding between the polypeptide and the test compound can be detected; and detecting binding between the polypeptide and the test compound.

As another aspect, the invention provides a method of identifying a compound that modulates the activity of a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2, and a functional fragment of any of the foregoing, comprising: contacting the polypeptide with a test compound under conditions whereby modulation of the activity of the polypeptide can be detected; and detecting modulation of the activity of the polypeptide.

As still a further aspect, the invention provides a method of identifying a compound that can modulate inflammatory responses, comprising: contacting a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2 and a functional fragment of any of the foregoing with a test compound under conditions whereby modulation of the activity of the polypeptide can be detected; and detecting modulation of the activity of the polypeptide, thereby identifying a compound that can modulate inflammatory responses.

As yet another aspect, the invention provides a method of identifying a compound that can modulate apoptosis, comprising: contacting a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2 and a functional fragment of any of the foregoing with a test compound under conditions whereby modulation of the activity of the polypeptide can be detected; and detecting modulation of the activity of the polypeptide, thereby identifying a compound that can modulate apoptosis.

As another aspect, the invention provides a method of identifying a compound that can modulate a Toll-like receptor pathway, comprising: contacting a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2 and a functional fragment of any of the foregoing with a test compound under conditions whereby modulation of the activity of the polypeptide can be detected; and detecting modulation of the activity of the polypeptide, thereby identifying a compound that can modulate the Toll-like receptor pathway.

These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic of the database and search strategies used to identify CATERPILLER family members.

Figure 2A depicts the genomic organization for known and some predicted members of the CATERPILLER family shown to scale. Black boxes represent exons. Unusually large introns are interrupted and their size indicated below in kilobase pairs. Exons with ambiguous positions are shown as grey boxes. The large 3' exons of Nod1 and Nod2 are 3' untranslated sequences.

Figure 2B shows twelve motifs defining the CATERPILLER NBD. Capital letters indicate residues (single letter code) that have a frequency greater than 50% or are invariant. Lower case letters indicate residues with frequency less than 50% but with a predominant characteristic (a = acidic, b = basic, h = hydrophobic, p = serine/threonine, r = aromatic). Asterisks indicate those residues used to define the NACHT family.^{1, 2, 3} Indicate NACHT motifs V, VI, and VIII respectively.

Figures 3A-3G show the alignment of nucleotide binding domains (NBD) of CATERPILLER family members using Clustal with minor manual adjustments.

Figure 4 illustrates a phylogenetic tree for NBDs. Deduced amino acid sequences from NBD exons were compared to one another using alignment and tree generation software in the DAMBE (Data Analysis in Molecular Biology and Evolution) software package. * indicates a predicted gene with unknown N-terminal sequences.

Figure 5 depicts the chromosomal location of each known or predicted sequence as indicated. For chromosomal locations with multiple sequences, the name order does not correspond to the ordering on the chromosome.

Figures 6A-C show the nucleotide and deduced amino acid sequences of full-length Monarch-1.

Figures 6D- F show the nucleotide and deduced amino acid sequences of Monarch-1 isoform II.

Figures 6G-I show the nucleotide and deduced amino acid sequences of Monarch-1 isoform III.

Figures 6J-L show the nucleotide and deduced amino acid sequences of Monarch-1 isoform IV.

Figure 7A shows the expression of Monarch-1 in separated human myeloid cell populations as determined by real-time PCR.

Figure 7B shows Monarch-1 expression in primary adherent cells after stimulation with DETA-NO, with $\text{TNF}\alpha$ or $\text{IFN}\gamma$ alone, or in combination as determined by real-time PCR. Monarch-1 expression was normalized to the expression of 18S rRNA. Student t-test was performed on control compared to treated cells (* = $p < 0.01$, + = $p < 0.05$). Three separate cell preparations were used and tested.

Figure 8 shows Monarch-1 expression in Hela lines stably transfected with Monarch-1 as determined by real-time PCR. Monarch-1 expression in total PBMCs was included for comparison. The level of Monarch-1 expression was normalized to the expression of GAPDH. Student t-test was performed on controls compared to stably transfected clones (* = $p < 0.01$).

Figure 9A shows the analysis of selected Monarch-1 regulated genes as determined by real-time PCR. Expression was normalized to the expression of 18S rRNA and shown as an exponential number. Student t-test was performed on controls compared to stable transfected clones (* = $p < 0.01$, + = $p < 0.05$).

Figure 9B shows human HLA surface expression on each of the Monarch-1 stable HeLa clones as determined by FACS analysis. In each

graph expression was compared to unstained (dotted line) and isotype control (solid line). Mean fluorescence intensity is displayed for each sample.

Figure 9C shows that Monarch-1 activates the HLA-B promoter-luciferase construct. Error bars represent the SEM of five separate experiments. Student t-test was performed on control compared to transfected clones (* = $p < 0.01$, + = $p < 0.05$).

Figure 10A shows the analysis of Monarch-1, HLA-B and HLA-G expression in Monarch-1 and mutant siRNA bulk cultures determined by real-time PCR. Expression was normalized to the expression of GAPDH mRNA and represented as fold over mutant control.

Figure 10B shows the analysis of Monarch-1, HLA-B and HLA-G expression in Monarch-1 siRNA clones as determined by real-time PCR. Three independent clones generated by stable transfection of the mutant siRNA are shown (represented as mut) and two independent clones generated by stable transfection of WT siRNA are shown (represented as siRNA). Expression was normalized to GAPDH. Data are represented as exponential numbers. Student t-test was performed on the average the control mutant clones compared to siRNA clones (* = $p < 0.01$, + = $p < 0.05$).

Figure 11 shows TLR activation reduces Monarch-1 expression. Human peripheral blood monocytes and granulocytes were treated with the different TLR2 and TLR4 activators and Monarch-1 expression was assessed by real-time PCR.

Figure. 12 shows that Monarch-1 is expressed during the LPS tolerant phase. Thp-1 monocytic cells were treated with LPS at the indicated time, and Monarch-1 expression was assessed as described in Figure 5 (top panel). After 18 hours, cells were washed and treated with media or LPS for 6 hours (bottom panel).

Figure 13 shows that Monarch-1 suppresses TLR signaling molecule NF- κ B activation. 293T cells were co-transfected with Monarch-1, a reporter construct bearing the NF- κ B binding site linked to luciferase, and MyD88, IRAK1, TRAF6, or TRAF2 was used to activate an NF- κ B reporter construct.

5 Open bars, transfected with a plasmid containing Monarch-1; shaded bars, transfected with an empty vector control.

Figure 14 shows that Monarch-1 is decreased in lung transplant BAL samples. Bronchiolar lavage samples from normal human subjects and lung

10 transplant patients were subjected to real-time PCR analysis for Monarch-1 expression.

Figure 15 shows that Monarch-1 enhances IL-10 induction. Stable Thp-1 clones expressing mutant siRNA targeting the Monarch-1 gene

15 (designated by M followed by clone number) and six clones expressing wild-type siRNA (designated by clone number) were left unstimulated (designated by C) or were stimulated with LPS (designated by L) for 24 hours. RNA was harvested and IL-10 expression examined by real-time PCR.

Figure 16 shows that Monarch-1 enhances TNF α induction. Stable Thp-1 clones expressing mutant siRNA targeting the Monarch-1 gene

20 (designated by M followed by clone number) and six clones expressing wild-type siRNA (designated by clone number) were left unstimulated (designated by C) or were stimulated with LPS (designated by L) for 3 hours. RNA was

25 harvested and TNF α expression examined by real-time PCR.

Figure 17 shows Monarch-1-regulated pro- and anti-inflammatory cytokine induction. Global analysis of cytokine protein expression in stable Thp-1 clones expressing mutant siRNA Monarch-1 oligo (designated by M

30 follows by clone number) and six clones expressing wild-type siRNA oligo targeting Monarch-1 expression (designated by clone number) were left unstimulated (designated by C) or were stimulated with LPS (designated by L)

for 48 hours. Supernatants were isolated and a Cytometric Bead Assay (CBA) was performed to assess cytokine production.

5 Figures 18A-C show the nucleotide and deduced amino acid sequences of a predicted mouse Monarch-1.

Figure 19 demonstrates the expression of mouse Monarch-1 in various mouse cell lines as determined by separated real-time PCR and in primary tissues by real-time PCR.

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Figure 20 shows the expression of mouse Monarch-1 in myeloid suppressor cells isolated from BALB/c mice with large primary mammary carcinomas (tumor bearing) or mice with metastasis wherein primary mammary tumors had been surgically removed (non-tumor bearing). Normal spleen RNA (naïve spleen) was included as a control.

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Figures 21A-C show the nucleotide and deduced amino acid sequences of predicted CATERPILLER 11.2.

20 Figures 21D-F show the nucleotide and deduced amino acid sequences of cloned CATERPILLER 11.2.

Figure 22 shows the genomic organization of CATERPILLER 11.2 with boxes representing exons.

25

Figure 23A shows that CATERPILLER 11.2 significantly inhibits NF- κ B activity. HeLa cells were transfected with 0.5 μ g of 3X NF κ BLuc and 1 μ g of either empty vector or CATERPILLER 11.2 in the presence or absence of 100 ng of vector or CMV-p65.

30

Figure 23B shows that CATERPILLER 11.2 does not inhibit activation of the AP1 luciferase reporter by transfected c-jun. HeLa cells were

transfected with 0.5 µg of AP1Luc and 1 µg of either empty vector or CATERPILLER 11.2 in the presence or absence of 100 ng of vector or c-jun.

Figure 24 shows that CATERPILLER 11.2 inhibits basal and CIITA-induced HLA-DR promoter activity. HeLa cells were transfected with 0.5 µg of HLA-DRLuc and 1 µg of either empty vector or CATERPILLER 11.2 in the presence or absence of 100 ng of vector or CIITA.

Figures 25A-B show the nucleotide and deduced amino acid sequences of predicted CATERPILLER 11.3.

Figures 25C-E show the nucleotide and deduced amino acid sequences of cloned CATERPILLER 11.3. Nucleotide sequence includes 5' initiator codon and 3' stop.

Figures 25F-G show the nucleotide and deduced amino acid sequences of a splice variant of cloned CATERPILLER 11.3.

Figure 26 depicts the genomic organization of human CATERPILLER 11.3. Human CATERPILLER 11.3 consists of 9 exons spanning 3561 bp. Exon 4 and 5 both contain a putative nucleotide binding domain. Exons 6 through 9 contain putative leucine-rich repeat regions (LRRs).

Figure 27 shows that CATERPILLER 11.3 inhibits Myd88-induced NF-κB induction. HEK293T cells were seeded into 96-well plates and transfected on the following day with 50 ng of pNF-κB-luc and pcDNA3HA-MyD88 plasmid together with increasing amounts (1 ng to 400 ng) of pcDNA3HA-11.3. After 24 hrs, cells were harvested, and luciferase activity was determined for each sample. All data are shown as the average of triplicates and expressed in relative light units (RLU). The first bar represents cells transfected with only pcDNA3 and NF-κB-luc reporter. 10 ng/ml human CATERPILLER 11.3 produced an almost 4 fold reduction in Myd88 stimulated

NF- κ B reporter and was completely abolished at 400 ng/ml CATERPILLER 11.3

Figure 28 shows that CATERPILLER 11.3 inhibits NIK-induced NF- κ B induction. HEK293T cells were seeded into 96-well plates and transfected on the following day with 50 ng of pNF- κ B-luc and pcDNA3HA-NIK plasmid together with 100 ng/ml of pcDNA3HA-11.3. After 24 hrs, cells were harvested, and luciferase activity was determined for each sample. All data are shown as the average of triplicates and expressed in relative light units (RLU). The first bar represents cells transfected with only pcDNA3 and NF- κ B-luc reporter. 100ng/ml human CATERPILLER 11.3 produced an almost 2 fold reduction in NIK stimulated NF- κ B luciferase reporter.

Figures 29A-C show the nucleotide and deduced amino acid sequences predicted CATERPILLER 16.1.

Figures 29D-G show the nucleotide and deduced amino acid sequences cloned CATERPILLER 16.1.

Figure 30A-D show the nucleotide and deduced amino acid sequence of NOD27. Underlined sequences denote exons not present in cloned CATERPILLER 16.1.

Figure 31A shows the expression of transcripts of a murine ortholog of CATERPILLER 16.1. CATERPILLER m16.1 transcript levels in murine cell lines were determined by real-time PCR analysis. The average of three analyses is shown. Mouse cell lines include fibroblast (NIH3T3), erythroid leukemia (MEL), melanoma (B16F10), T cell (EL4), B cell (18.81, mature B), monocytic (WEHI3, J774A.1, P388D1), macrophage (RAW264), and primary bone marrow (BM). 18s RNA levels were quantitated and used as an internal standard for each sample.

Figure 31B shows resting levels of CATERPILLER m16.1 transcript in tissues and cells harvested from a saline-perfused mouse as determined by real-time PCR analysis. Data from three analyses are shown. The highest expression is seen in peripheral blood lymphocytes (PBL), spleen, thymus (thy), liver, and lung.

Figure 31C shows that the expression of CATERPILLER m16.1 in primary macrophage increases with LPS stimulation. Three mice were injected (i.p.) with thioglycolate. Five days after injection, the peritoneal macrophage were isolated and then treated with LPS for 1, 3 or 6 hours in comparison to control macrophages that were untreated for the same time points. An increase in expression of CATERPILLER m16.1 is seen at 3 and 6 hours of LPS stimulation.

Figure 32 shows the expression of a murine ortholog of CATERPILLER 16.1 in virally-induced arthritic tissues. RNA was prepared from skeletal muscle samples from arthritic joints of mice injected with Ross River Virus for various times (24 – 144 hours post-injection). Fold induction of CATERPILLER m16.1 expression in the muscle was determined by real-time PCR analyses through a comparison of viral-infected samples (n=3) to mock-infected samples (n=3) at each time point indicated. Two sets of data at each time point are presented. The expression is greatly induced at 24 hours, while the induction subsides between 48 and 96 hours, and then increases again at 120 and 144 hours.

Figure 33A shows that the expression of a murine ortholog of CATERPILLER 16.1 (m16.1) in transplanted heart tissues is greatly increased. RNA from mismatched heart tissues (allo) vs. genetically identical (iso) tissues harvested 7 days (7d) after transplantation was analyzed by real-time PCR. Samples from three transplanted tissues (A, B, C) are shown. 18s RNA levels were quantitated and used as an internal standard for each sample.

Figure 33B shows that CATERPILLER m16.1 expression in transplanted kidney tissues is greatly enhanced. Kidneys transplanted into genetically identical hosts (iso) or MHC-mismatched hosts (allo) were harvested at 7, 15, or 100 days (d) post transplantation and RNA was
5 analyzed by real-time PCR. Multiple samples (n=2-5) for each group were analyzed. Kidney transplant recipients of MHC-mismatched organs surviving for 100 days (100d allo) have surpassed the critical inflammatory processes that normally result in graft rejection. 18s RNA levels were quantitated and used as an internal standard for each sample.

10

Figures 34A-C show the nucleotide and deduced amino acid sequences predicted CATERPILLER 16.2.

Figures 34D-F show the nucleotide and deduced amino acid
15 sequences cloned CATERPILLER 16.2.

Figure 35A shows the expression of human CATERPILLER 16.2 mRNA in various human cell lines as determined by separated real-time PCR. Representative of two experiments.

20

Figure 35B shows the expression of CATERPILLER 16.2 in human total PBMC populations, PBMCs separated based on adherence, or the indicated human cell lines as determined by real-time PCR. CATERPILLER 16.2 expression was normalized to the expression of 18S rRNA.
25 Representative of two experiments.

Figure 36A shows the expression of mouse CATERPILLER 16.2 mRNA in various mouse cell lines as determined by separated real-time PCR. Average of three experiments.

30

Figure 36B shows mouse CATERPILLER 16.2 expression in perfused mouse tissues or peripheral blood by real-time PCR. CATERPILLER 16.2 expression was normalized to the expression of 18S rRNA. Three real-time

runs were performed and are shown.

Figure 36C shows mouse CATERPILLER 16.2 expression in peritoneal macrophage treated with LPS for up to 3 hours compared to control cells (C) not treated with LPS for the same time. Three sets of treated and untreated macrophage were subjected to real-time PCR analysis.

Figure 37A shows that CATERPILLER 16.2 inhibits NF- κ B induction. HEK293T cells were seeded into 96-well plates and transfected on the following day with 50 ng of pNF- κ B-luc reporter gene plasmid together with increasing amounts (100 ng to 400 ng) of pcDNA3Fg-16.2 and the indicated wells were stimulated with TNF α . The two last bars represent a control in which 50 ng of p53-luc reporter gene plasmid, 200 ng of p53-encoding plasmid and 400 ng of pcDNA3Fg-16.2 were transfected. After 36 hours, cells were harvested, and luciferase activity was determined for each sample. *Numbers* indicate fold induction of the NF- κ B reporter gene above base-line.

Figure 37B shows that CATERPILLER 16.2 inhibits AP-1 induction. HEK293T cells were seeded into 96-well plates and transfected on the following day with 50 ng of AP-1-luc reporter gene plasmid together with increasing amounts (100 ng to 400 ng) of pcDNA3Fg-16.2 and the indicated wells were stimulated with PMA β . After 36 hours, cells were harvested, and luciferase activity was determined for each sample. *Numbers* indicate fold induction of the AP-1 reporter gene above base-line.

Figure 38A shows the induction of CIAS1 in primary human monocytes. Adherence-purified human monocytes were stimulated as indicated for 1 hour before lysis, RNA preparation and analysis.

Figure 38B shows that LPS (200 ng/ml) rapidly induces CIAS1.

Figure 38C shows induction of CIAS1 in monocytes pretreated with inhibitors of the MAPK, p38, and PI3K pathways for 20 minutes prior to LPS stimulation.

5 Figure 39A shows FLAG®-tagged full-length CIAS1 (Fg CIAS1) and the shorter, naturally occurring isoforms missing exon 4 or exons 4 and 6.

Figure 39B shows that all the CIAS1 constructs inhibited NF- κ B-luciferase activation by TNF α in a dose-dependent fashion

10

Figure 39C shows that FgCIAS1 inhibits the ability of transfected p65 to stimulate NF- κ B-luciferase in a dose-dependent fashion (left panel). FgCIAS1 does not affect p53 function (right panel). Representative of three or more assays performed in triplicate \pm SEM.

15

Figure 40 shows that FgCIAS1 inhibits TNF α -induced nuclear translocation of p65. Localization of p65 in CIAS1-positive cells was scored qualitatively as described in EXAMPLE 7. p65 localization was scored as primarily nuclear (N), evenly nuclear/cytoplasmic (N/C) or primarily cytoplasmic (C) p65. Quantitative data shown are the composite percentages from three individual experiments.

20

Figure 41A depicts deletion mutant constructs of CIAS1.

25 Figure 41B shows the relative activation of NF- κ B luciferase in HeLa cells transfected with 1.5 μ g/well of pcDNA3 (control) or the indicated construct, followed by transfection with p65, 24 hours post-transfection as described in EXAMPLE 7. Values are means of three experiments \pm SEM.

30 Figures 42A-B show the nucleotide and deduced amino acid sequences of a pyrin only containing CIAS1 protein.

Figures 42C-E show the nucleotide and deduced amino acid sequences of a pyrin/NBD containing CIAS1 protein.

Figures 42F-H show the nucleotide and deduced amino acid sequences of a NBD/LRR containing CIAS1 protein.

Figures 42I-J show the nucleotide and deduced amino acid sequences of a LLR containing CIAS1 protein.

Figures 42K-M show the nucleotide and deduced amino acid sequences of a FgCIAS1 Del4 isoform.

Figure 43 shows that CIAS-1 inhibits the ability of overexpressed CIITA to activate DR-Luciferase in HeLa cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will now be described in more detail with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right, unless specifically indicated otherwise. Nucleotides and amino acids are represented herein in the manner

recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by either the one-letter code, or the three letter code, both in accordance with 37 CFR §1.822 and established usage.

5 Except as otherwise indicated, standard methods known to those skilled in the art may be used for cloning genes, amplifying and detecting nucleic acids, and the like. Such techniques are known to those skilled in the art. See, e.g., SAMBROOK *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed. (Cold Spring Harbor, NY, 1989); F. M. AUSUBEL *et al.* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Green Publishing
10 Associates, Inc. and John Wiley & Sons, Inc., New York).

I. Definitions.

As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms
15 as well, unless the context clearly indicates otherwise.

The term "modulate," "modulates" or "modulation" refers to enhancement (e.g., an increase) or inhibition (e.g., a reduction) in the specified activity.

20 The term "enhance," "enhances," "enhancing" or "enhancement" refers to an increase in the specified parameter (e.g., at least about a 1.25-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, twelve-fold, or even fifteen-fold or more increase).

The term "inhibit" or "reduce" or grammatical variations thereof as used herein refers to a decrease or diminishment in the specified activity of at least
25 about 25%, 35%, 40%, 50%, 60%, 75%, 80%, 90%, 95% or more. In particular embodiments, the inhibition or reduction results in little or essentially no detectable activity (at most, an insignificant amount, e.g., less than about 10% or even 5%).

A "therapeutically effective" amount as used herein is an amount that
30 provides some improvement or benefit to the subject. Alternatively stated, a "therapeutically-effective" amount is an amount that will provide some alleviation, mitigation, or decrease in at least one clinical symptom in the subject (e.g., reduced inflammation, sepsis, or tumor size). Those skilled in

the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

By the terms "treat," "treating" or "treatment of," it is intended that the severity of the subject's condition is reduced or at least partially improved or modified and that some alleviation, mitigation or decrease in at least one clinical symptom is achieved.

As used herein, a "vector" or "delivery vector" can be a viral or non-viral vector that is used to deliver a nucleic acid to a cell, tissue or subject.

A "recombinant" vector or delivery vector refers to a viral or non-viral vector that comprises one or more heterologous nucleotide sequences (*i.e.*, transgenes), *e.g.*, two, three, four, five or more heterologous nucleotide sequences.

As used herein, the term "polypeptide" encompasses both peptides and proteins, unless indicated otherwise.

A "fusion protein" is a polypeptide produced when two heterologous nucleotide sequences or fragments thereof coding for two (or more) different polypeptides not found fused together in nature are fused together in the correct translational reading frame. Illustrative fusion polypeptides include fusions of a CATERPILLER polypeptide (or a fragment thereof) to all or a portion of glutathione-S-transferase, maltose-binding protein, or a reporter protein (*e.g.*, Green Fluorescent Protein, β -glucuronidase, β -galactosidase, luciferase, *etc.*), hemagglutinin, c-myc, FLAG epitope, *etc.*

As used herein, a "functional" polypeptide or "functional fragment" is one that substantially retains at least one biological activity normally associated with that polypeptide. In particular embodiments, the "functional" polypeptide or "functional fragment" substantially retains all of the activities possessed by the unmodified peptide. By "substantially retains" biological activity, it is meant that the polypeptide retains at least about 50%, 60%, 75%, 85%, 90%, 95%, 97%, 98%, 99%, or more, of the biological activity of the native polypeptide (and can even have a higher level of activity than the native polypeptide). A "non-functional" polypeptide is one that exhibits little or essentially no detectable biological activity normally associated with the

polypeptide (e.g., at most, only an insignificant amount, e.g., less than about 10% or even 5%).

As used herein, an "isolated" nucleic acid (e.g., an "isolated DNA") means a nucleic acid separated or substantially free from at least some of the other components of the naturally occurring organism or virus, such as for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the nucleic acid.

Likewise, an "isolated" polypeptide means a polypeptide that is separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide. In particular embodiments, the "isolated" polypeptide is at least about 1%, 5%, 10%, 25%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or more pure (w/w). In other embodiments, an "isolated" polypeptide indicates that at least about a 5-fold, 10-fold, 25-fold, 100-fold, 1000-fold, 10,000-fold, or more enrichment of the protein (w/w) is achieved as compared with the starting material.

By the term "express" or "expression" of a nucleic acid coding sequence, in particular a CATERPILLER coding sequence, it is meant that the sequence is transcribed, and optionally, translated. Typically, according to the present invention, expression of a CATERPILLER coding sequence will result in production of the CATERPILLER polypeptide. The entire expressed polypeptide or fragment can also function in intact cells without purification.

The term "about," as used herein when referring to a measurable value such as an amount of polypeptide, dose, time, temperature, enzymatic activity or other biological activity and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

II. The CATERPILLER Gene Family.

The inventors have discovered and characterized a new family of genes based on the presence of identified protein motifs. The few previously-known genes that belong to this family have roles in inflammation or apoptosis or both, and most are linked to immunologic diseases.

This new gene family comprises at least 22 members. All of the genes classified within this family contain a combination of two or more landmark amino acid stretches. These include a nucleotide-binding domain (NTB) and a leucine-rich repeat (LRR) region. In addition, a majority of the genes have a pyrin domain, a recently described domain associated with Mediterranean Fever. Others have the Caspase recruitment domain (CARD), or an unknown N-terminal domain(s). The inventors have designated this new family as the CATERPILLER (CARD, Transcription Enhancer, R(purine)-binding, Pyrin, Lots of Leucine Repeats) gene family.

The CATERPILLER family is implicated in a variety of disorders. To date, the few known members of the CATERPILLER family have been linked to either apoptosis or autoinflammatory/immune diseases, suggesting that the newly identified genes may also be important for apoptosis and inflammatory diseases. These previously known genes include CIITA (a master regulator of MHCII expression), NOD1 (apoptosis), NOD2 (inflammatory bowel disease and Blau's syndrome), CIAS1/cryopyrin (familial cold autoinflammatory syndrome), and DEFCAP (apoptosis). In fact, the inventors have isolated the nucleic acids, determined the sequences, and characterized several of these newly identified family members and have shown that, in fact, they are also involved in inflammatory processes and cell survival. Further, analysis of one of the known genes, CIAS1/cryopyrin, has indicated a new function in down-regulating important modulators of immune function.

A brief description of several members of the CATERPILLER family (Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1 and CATERPILLER 16.2) is provided below.

Monarch-1 (also known as Caterpillar 19.3): The present investigations have described the entire cDNA sequence (SEQ ID NO:1; Accession No. AY116204) of a Caterpillar family member named Monarch-1, which has pyrin, nucleotide-binding (NBD) and leucine-rich repeat (LRR) domains (amino acid sequence; SEQ ID NO:2). Three naturally occurring isoforms (nucleotide sequences: SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:7 and corresponding amino acid sequences: SEQ ID NO:4, SEQ ID NO:6 and SEQ

ID NO:8, respectively; see *also* Accession Nos. AY116205, AY116206 and AY116207) of Monarch-1 have been identified. Monarch-1 is located on human chromosome 19q13, in the multiple sclerosis susceptibility region. A prominent downstream effect of Monarch-1 is induction of both classical and non-classical class I MHC genes and LMP7. The present investigations have further demonstrated that:

(1) Monarch-1 is expressed in monocytic and myeloid cells including granulocytes (neutrophils and eosinophils), monocytes and dendritic cells.

(2) Monarch-1 is reduced by activators of the Toll-like receptor (TLR), that recognize bacterial, fungal and viral products.

(3) All of the Monarch-1 isoforms induce class I MHC genes in cell.

(4) Monarch-1 reduces NF- κ B and AP-1 activity, which are important transcription factors involved in both inflammatory responses and cell survival.

(5) A predicted mouse Monarch-1 (GenBank Accession No. XM_142563; the disclosure of which is incorporated herein by reference in its entirety) has been identified, and found to be expressed by immune cells. Mouse Monarch-1 shares about 82% nucleotide sequence similarity with the human sequence.

(6) Human Monarch-1 is part of the endotoxin tolerant pathway and its expression is maintained when cells are tolerant to endotoxin. Endotoxin tolerance is a phenomenon in septic patients, in which tolerance is developed to bacterial products.

(7) Monarch-1 inhibits cellular responses induced by endotoxin from bacteria.

(8) Monarch-1 inhibits IFI16, an interferon responsive protein.

(9) Monarch-1 causes changes in cytokine (IL-6, IL1 β , and IL-10) expression, which are believed to be important for all immune and inflammatory responses. The most dramatic change is in IL-6, which is a strong pro-inflammatory cytokine. IL-1 is also a pro-inflammatory cytokine, while IL-10 typically directs a pro-inflammatory response important in asthma and allergies, among other diseases, and can be immunosuppressive of T cell activation.

(10) Interference RNA has been made which inhibits the function of

Monarch-1 and shows its function in the enhancement of class I MHC gene expression and cytokine production.

(11) Monarch-1 interacts with a host of proteins, which can be exploited to interfere or enhance the function of these proteins. These include
5 tubulin, vimentin, hsp-70, TNF- α , CARD10, TRAF6, NIK and CIAS1.

Altogether, these data indicate that Monarch-1 is a positive regulator of MHC-I and IL-6/IL-10/IL-1 β expression in myeloid monocytic cells, and a target of the TLR pathway. In addition it appears that Monarch-1 represents a
10 new pathway for MHC-I induction separate from the TNF- α and IFN- γ pathway.

CIAS1 (Cold-induced autoinflammatory syndrome1): CIAS1 was first described as the genetic basis for the cold-induced autoinflammatory
15 syndrome and the Muckle-Wells syndrome. The investigations herein describe the activity of CIAS1 in the suppression of NF- κ B and CIITA function. NF- κ B controls inflammatory responses and apoptosis while CIITA controls the expression of major histocompatibility complex (MHC) class II genes, important in the stimulation of T lymphocytes. In addition, the inventors have
20 found that the full length human CIAS1 (Accession No. NM_004895; the disclosure of which is incorporated herein by reference in its entirety) or either of two shorter, naturally occurring isoforms (Accession No. AY092033 [lacking exons 4 and 6], the disclosure of which is incorporated herein by reference in its entirety, and a novel isoform disclosed herein that lacks exon 4 but has
25 exon 6; **SEQ ID NO:148** [nucleotide sequence] and **SEQ ID NO:149** [amino acid sequence]) dramatically inhibit TNF α -induced activation of NF- κ B reporter activity. Transcriptional activity of exogenous NF- κ B p65 was also blocked by CIAS1. Studies with a truncated protein (nucleotide sequence, **SEQ ID NO:33**; amino acid sequence, **SEQ ID NO:34**) that contains the
30 nucleotide-binding (NTB) and leucine-rich repeat (LRR) regions, but not the pyrin domain, of CIAS1 indicate that the NTB and LRR regions are sufficient for this inhibition. CIAS1 also suppresses TNF α -induced nuclear translocation of endogenous p65. These data suggest CIAS1 may act as a key negative

regulator of inflammation, induced to dampen NF- κ B-dependent pro-inflammatory and pro-survival signals. In addition, its suppressive effects on CIITA indicate a function in the downregulation of MHC-II protein, important for T cell stimulation. MHC-II has a variety of roles in autoimmune diseases and transplantation rejection. The inventors have found that ligands recognized by multiple Toll-Like Receptors (TLRs) induce CIAS1 gene expression in primary human monocytes, utilizing the MAPK/p38 but not PI3K signaling pathways.

A mouse homolog of CIAS1 is found at Accession No. NM_145827 (the disclosure of which is incorporated herein by reference in its entirety).

Mutations in CIAS1 have been linked recently to three chronic autoinflammatory disorders. These observations point to an important role for CIAS1 in regulating inflammatory processes. The locus responsible for the chronic, autosomal-dominant autoinflammatory periodic fever syndromes Familial Cold Urticaria (FCU), and Muckle-Wells Syndrome was found on chromosome 1q44 with pathology-associated mutations present in the CIAS1 gene (Hoffman et al., (2001) *Nat. Genet.* **29**:301). Common symptoms of these genetic disorders include periodic fever, rash, arthralgia, and conjunctivitis. Mutations of CIAS1 were also found in the chronic infantile neurologic, cutaneous, articular (CINCA) syndrome (Feldmann et al., (2002) *Am. J. Hum. Genet.* **71**:198).

CIAS1 contains an amino terminal Pyrin domain, a centrally located predicted NBD, and numerous LRR motifs at its carboxy terminus. The pyrin domain of CIAS1 is highly homologous to its namesake, the Pyrin protein encoded by the *MEFV* gene. Recent published reports provide evidence that CIAS1 may be involved in the regulation of IL-1 generation and NF- κ B activation (Manji et al., (2002) *J. Biol. Chem.* **277**:11570; Wang et al., (2002) *J. Biol. Chem.* **277**:29874), placing *CIAS1* in the inflammatory cascade.

The pro-inflammatory signaling program in myeloid cells leads to activation of the cytokines IL-1, IL-6, IL-8, and TNF α , as well as reactive oxygen species and other molecules through a number of steps culminating in transcriptional activity (reviewed in Suzuki et al., (2002) *Trends Immunol.* **23**:503). Initiation of the signaling cascade frequently begins with cell surface-

expressed TLRs sensing a variety of pathogenic products, stimulation of the IL-1 receptor, or crosslinking of the TNF α receptor. These diverse signaling pathways initially utilize an assortment of signaling intermediates (Zhang et al., (1999) *J. Biol. Chem.* **274**:7611; Suzuki et al., (2002) *Trends Immunol.* **23**:503; Chen et al., (2002) *Science* **296**:1634) but converge downstream to induce activity of the transcription factors NF- κ B, AP-1 and others.

TNF α stimulation leads to phosphorylation, ubiquitination, and degradation of I κ B α , liberating the p50 and p65 subunits of NF- κ B. The p65 subunit is phosphorylated and enters the nucleus to initiate transcription of various inflammatory genes. The present investigations have evaluated the effects of CIAS1 on TNF α signaling since TNF α is widely regarded as one of the most potent inflammatory stimulants.

The onset of inflammation is a central response to pathogens, autoimmune antigens and injury. Yet the resolution and down-regulatory phase of this response to prevent irrevocable damage is of equal importance. The present inventors have shown that CIAS1 is induced by a number of pathogenic molecules that can activate diverse TLRs, but that it can be a negative regulator of TNF α induced NF- κ B activation. This inhibition is concentration-dependent and occurs by disallowing nuclear translocation of the p65 subunit of NF- κ B. Previously, CIAS1 has been suggested to play a role in the generation of IL-1 and activation of NF- κ B, but only when expressed in concert with the adaptor molecule apoptosis-associated speck-like protein (ASC) (Manji et al., (2002) *J. Biol. Chem.* **277**:11570). The data reported herein reveal that CIAS1 alone reduces TNF α and NF- κ B responses. Together, these studies suggest that the balance of ASC and CIAS1 determines the extent of inflammatory responses, and that alone, either may serve as an important suppressor molecule. It is interesting to note that NF- κ B nuclear translocation is routinely detectable within 10-30 minutes after cell activation while increases in CIAS1 RNA are observed 30-60 minutes after stimulation. One possibility is that CIAS1 is induced to limit the extent of the pro-inflammatory cytokine cascade, preventing hyper-inflammation seen in autoinflammatory syndrome patients. In this scenario, mutations in CIAS1

lead to dysfunctional inhibition and prolonged, exaggerated inflammatory responses.

Other proteins with similar CARD and/or Pyrin domains have been shown to activate NF- κ B *in-vitro*. One example is Nod1, proposed to induce NF- κ B activity by bringing the CARD-containing kinase RICK in close proximity with the gamma regulatory subunit of IkappaB kinase (IKK) (Inohara et al., (2000) *J. Biol. Chem.* **275**:27823). Another report describes a complex of CARD- and Pyrin-containing proteins assembling to elicit processing of pro-IL-1 β , a signaling platform termed the 'inflammasome' (Martinon et al., (2002) *Mol. Cell* **10**:417). In contrast, the CARDINAL / TUCAN and PAN2 proteins possess NF- κ B suppressor activity (Bouchier-Hayes et al., (2001) *J. Biol. Chem.* **276**:44069; Fiorentino et al., (2002) *J. Biol. Chem.* **277**:35333). The emerging view is of a complex balance between pro and anti-inflammatory molecules that in the proper context serve to initiate, amplify, or suppress inflammatory processes.

As mentioned above, the NBD and LRR regions of CIAS1 are sufficient for the inhibitory activity of the full-length protein. A curious finding is the stimulatory activity of the CIAS1 Pyrin domain alone. Without wishing to be bound by any particular theory of the invention, it appears that the Pyrin domain expressed alone may artificially act as an oligomerization domain bringing NF- κ B activating molecules together as has been proposed for Nod1. On the other hand, positive cooperation of CIAS1 with ASC also involves the pyrin domain (Manji et al., (2002) *J. Biol. Chem.* **277**:11570).

CATERPILLER 11.2: The nucleotide (SEQ ID NO:11) and amino acid (SEQ ID NO:12) sequences of another member of the CATERPILLER family, CATERPILLER 11.2, were predicted based on the presence of putative pyrin, nucleotide-binding (NBD) and leucine-rich (LRR) domains. CATERPILLER 11.2 is located on human chromosome 11. The inventors have shown that CATERPILLER 11.2 reduces the function of NF- κ B, an important transcription factor involved in both inflammatory responses and cell survival. CATERPILLER 11.2 expression is primarily found in hematopoietic cell lines. The reduction of NF- κ B function by CATERPILLER 11.2 suggests that

CATERPILLER 11.2 is important in the control of immunity, gene expression and cell survival because NF- κ B controls all these processes. In addition, CATERPILLER 11.2 suppresses the expression of the class II Major Histocompatibility Complex (MHC-II) promoter. Proper MHC-II expression is important for immune recognition to elicit T cell responses against all pathogens and antigens.

The inventors have cloned the human CATERPILLER 11.2; the nucleotide and amino acid sequences are shown as **SEQ ID NO:13** and **SEQ ID NO:14**, respectively. The cloned nucleotide sequence differs from the predicted sequence as follows. The cloned sequence contains an additional (non-predicted) exon from approximately nucleotide (nt) 1959 to nt 2123. Further, the cloned sequence lacks a predicted exon from approximately nt 2124 to nt 2292 based on the predicted sequence. The cloned sequence lacks 222 nucleotides from the 3' end relative to the prediction. No evidence has been obtained to date to suggest that the additional 222 nucleotides are present in the coding message.

CATERPILLER 11.3:

The predicted nucleotide (**SEQ ID NO:15**) and amino acid (**SEQ ID NO:16**) sequences of another family member, human CATERPILLER 11.3, were determined based on the presence of putative nucleotide-binding (NBD) and leucine-rich (LRR) domains. The inventors have further cloned and characterized the human CATERPILLER 11.3 gene (nucleotide sequence, [**SEQ ID NO:17**]; amino acid sequence, [**SEQ ID NO:18**]) and a splice variant (nucleotide sequence, [**SEQ ID NO:19**]; amino acid sequence, [**SEQ ID NO:20**]). The CATERPILLER 11.3 gene resides at 11q23 on human chromosome 11 and contains as many as 9 exons based on both bioinformatics predictions as well as sequence data obtained from cloning the CATERPILLER 11.3 gene. Assembly of PCR products from the T cell line Jurkat yielded an approximately 3.6 kilobase pair (kb) insert containing both the initiator codon (ATG) and an in-frame stop codon that precedes a 3'UTR and poly-adenylation site.

CATERPILLER 11.3 is widely expressed and appears to be pro-inflammatory, at least in certain cell types including T-regulatory cells, suggesting that CATERPILLER 11.3 may be important for adaptive immunity (e.g., important for vaccines and transplantation). Expression of

5 CATERPILLER 11.3 is also markedly increased (about 10X) in a mouse model for inflammatory bowel disease.

CATERPILLER 16.1: Another member of the CATERPILLER family, CATERPILLER 16.1, was identified based on the presence of nucleotide

10 binding and leucine rich domains. CATERPILLER 16.1 is located on human chromosome 16q13 and is situated between *CTEP* and *CPNE2*. The predicted nucleotide sequence is shown as **SEQ ID NO:21** and the predicted amino acid sequence is shown as **SEQ ID NO:22**. A CATERPILLER 16.1 sequence has been cloned and characterized (nucleotide sequence, [**SEQ ID**

15 **NO:23**]; amino acid sequence, [**SEQ ID NO:24**]).

Expression of CATERPILLER 16.1 is found in cell lines and primary human cells of hematopoietic origin (but not restricted to these cell types), including B and T lymphocytes, monocytes and granulocytes. CATERPILLER 16.1 expression is affected by activation stimuli in Jurkat T cells (a human T

20 lymphocyte cell line) and differentiation stimuli in HL-60 cells (a human promyelocytic cell line). CATERPILLER 16.1 is implicated in both differentiation and activation of certain cell types are implicated in host responses to pathogens or the regulation of autoimmune diseases and/or cancer or precancerous conditions. CATERPILLER 16.1 expression is

25 dramatically increased (about 100-1000X) in the affected tissues of inflammatory disease models of arthritis, transplantation, CNS inflammatory disease, and Crohn's disease. Moreover, CATERPILLER 16.1 maps within the Crohn's susceptibility region.

NOD27, which shares structural similarity with CATERPILLER 16.1,

30 has recently been cloned and identified (GenBank Accession No. AF389420; *Biochem. Biophys. Res. Commun.* 14:302 (2003); the disclosures of which are incorporated by reference in their entireties).

CATERPILLER 16.2: The predicted nucleotide (SEQ ID NO:25) and amino acid (SEQ ID NO:26) sequences of another member of the CATERPILLER family, CATERPILLER 16.2, were predicted based on the presence of putative nucleotide-binding (NBD) and leucine-rich repeat (LRR) domains. This gene is located on human chromosome 16. The inventors have cloned and characterized the entire coding sequence for human CATERPILLER 16.2 (nucleotide sequence, [SEQ ID NO:27]; amino acid sequence, [SEQ ID NO:28]). The cloned sequence of CATERPILLER 16.2 is identical to the predicted sequence from nucleotides 286 – 2217. Note that nucleotide 286 of the cloned sequences is the position of the initiation methionine in the predicted version. The 3' end of the cloned sequence, nucleotides 2218-3489 differs from the predicted version.

CATERPILLER 16.2 has been shown to reduce the function of two important transcription factors, NF- κ B and AP-1, which are involved in both inflammatory responses and cell survival. CATERPILLER 16.2 expression is primarily found in peripheral blood leucocytes, and is reduced by bacterial products that activate the Toll-like receptor (TLR) pathway, the recognition receptors for bacteria, virus, fungus and other pathogens. This observation suggests that 16.2 is part of the TLR pathway.

CATERPILLER Nucleic acids, polypeptides, expression vectors, host cells and antibodies.

In representative embodiments, the invention provides isolated nucleic acids encoding a CATERPILLER polypeptide (or a functional fragment thereof) as well as the isolated CATERPILLER polypeptides (or a functional fragment thereof). The CATERPILLER nucleic acids and polypeptides of the invention encompass sequences from any species of interest (e.g., mammalian [human, simian, mouse, rat, lagomorph, bovine, ovine, caprine, porcine, equine, feline, canine, *etc.*], insect, yeast, avian, plants, *etc.*) as well as allelic variations, isoforms, splice variants and the like (e.g., Monarch-1 encompasses the splice variants and CATERPILLER 11.3 encompasses the splice variant disclosed herein). The CATERPILLER nucleic acids and polypeptides also include modifications that result in functional polypeptides.

Indicia of "functional" CATERPILLER polypeptides include those measures disclosed herein (e.g., in the working Examples) as well as other assays and techniques known in the art for determining inflammatory response, apoptosis, response to pathogens, NF- κ B activity, etc. and other activities associated with the function of the specific CATERPILLER polypeptide. Representative assays include NF- κ B and AP-1 reporter assays, evaluation of activation and/or production of NF- κ B/AP-1 by inducers such as TNF α , IL-1 or TLR signaling molecules, evaluation of cytokine expression and/or profiles and the like.

Thus, as one aspect, the invention provides an isolated nucleic acid encoding Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1 or CATERPILLER 16.2. In exemplary embodiments, the isolated nucleic acid comprises, consists essentially of, or consists of the nucleotide sequences of **SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:33 or SEQ ID NO:148.**

Isolated nucleic acids of this invention include RNA, DNA (including cDNAs) and chimeras thereof. The isolated nucleic acids can further comprise modified nucleotides or nucleotide analogs.

In other embodiments, the invention provides a nucleic acid that encodes a functional fragment of a Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1 or CATERPILLER 16.2 polypeptide (e.g., a fragment of **SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:33 or SEQ ID NO:148** and other fragments disclosed herein). Such nucleic acids will typically comprise at least about 30, 40, 50, 60, 80, 100, 125, 150, 200, 250 300, 500, 1000 or 1500 contiguous bases of a nucleotide sequence encoding the indicated CATERPILLER polypeptide and encodes a functional fragment thereof.

As yet a further aspect, the invention provides an isolated Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1 or CATERPILLER 16.2 polypeptide. In exemplary embodiments, the polypeptide comprises, consists essentially of, or consists of the amino acid

sequence of **SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:34 or SEQ ID NO:149.**

The CATERPILLER polypeptides of the invention also include
5 functional portions or fragments of a Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1 or CATERPILLER 16.2 polypeptide (e.g., functional fragments of **SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:34 or SEQ ID NO:149** and other
10 polypeptide fragments disclosed herein). The length of the fragment is not critical as long as it substantially retains the biological activity of the polypeptide. Illustrative fragments comprise at least about 10, 12, 15, 18, 20, 25, 30, 35, 40, 50, 100, 200, 300, 500 or 1000 contiguous amino acids of a CATERPILLER polypeptide.

15 The present inventors have discovered that the CATERPILLER family members comprise a nucleotide binding domain (NBD), a leucine rich repeat (LRR) region and, optionally, a pyrin domain. In particular embodiments, the invention provides a functional fragment of a CATERPILLER polypeptide (e.g., Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3,
20 CATERPILLER 16.1 or CATERPILLER 16.2) comprising the NBD or the LRR region, or both (and nucleic acids encoding the same). For those CATERPILLER polypeptides that contain pyrin domains, the functional fragment can further comprise a pyrin domain. As one representative example, the nucleic acid (**SEQ ID NO:33**) and amino acid (**SEQ ID NO:34**)
25 sequences of a functional fragment of CIAS1 comprising the NBD and LRR regions are disclosed herein. In other embodiments, the functional fragment of the CATERPILLER polypeptide comprises a pyrin domain, which has been reported to be important in the activity of the FMF (familial Mediterranean fever) protein, see Chae et al., "Targeted disruption of pyrin in the FMF
30 (familial Mediterranean fever) protein caused increased sensitivity to endotoxin and defective macrophage apoptosis," *Mol. Cell.* 11:591 (2003); the disclosure of which is incorporated herein in its entirety). In still other embodiments, the functional fragment of the CATERPILLER polypeptide

comprises the CARD domain, which is similar to caspase activation and recruitment domains that can lead to apoptosis (see, Bouchier-Hayes et al., (2002) *EMBO Rep.* 3:616).

With particular respect to Monarch-1, in particular embodiments, a functional fragment of Monarch-1 comprises the N-terminus of the protein (e.g., including the initiator methionine), and the corresponding nucleic acid comprises the 5' end of the coding sequence (e.g., including the initiator codon). In other particular embodiments, the functional fragment comprises at least about 20, 30, 50, 100 or 150 contiguous amino acids from the N-terminal portion of the protein from amino acid 1 to about amino acid 650 or 700 (see, e.g., **SEQ ID NO:2**).

With respect to CATERPILLER 11.3, in particular embodiments, the functional fragment comprises the amino-terminus of the protein (and the nucleic acid encoding the fragment comprises the 5' coding region and initiation codon). In other embodiments, the functional CATERPILLER 11.3 fragment comprises at least about 20, 30, 50, 100 or 150 contiguous amino acids from the N-terminal region from the initiator Met to about amino acid 300 or 350 (see, e.g., **SEQ ID NO:18** and **SEQ ID NO:20**). With respect to nucleic acids encoding a functional fragment of CATERPILLER 11.3, in particular embodiments, the isolated nucleic acid comprises at least about 20, 40, 50, 100, 150, 200, 250 or 500 or more contiguous bases from nucleotide 1 to about nt 1117 (see, e.g., **SEQ ID NO:17** and **SEQ ID NO:19**). In particular embodiments, the isolated nucleic acid comprises essentially all of nt 1 to nt 1117.

In other embodiments, the full-length or functional fragment of a CATERPILLER 16.1 polypeptide comprises a Leu at amino acid position 132 and/or an Arg at amino acid position 177 (see, e.g., **SEQ ID NO:24**).

In still other representative embodiments, a functional fragment of a CATERPILLER 16.2 polypeptide comprises the N-terminus of the polypeptide (i.e., the initiator codon). In other embodiments, the nucleic acid encoding the functional fragment of the CATERPILLER 16.2 polypeptide comprises at least about 20, 40, 50, 100, 150, 200, 250, 500 contiguous nucleotides of the 5' 1900 nucleotides of the CATERPILLER 16.2 coding sequence (see, e.g.,

SEQ ID NO:27). In other embodiments, the nucleic acid encoding the functional fragment of the CATERPILLER 16.2 polypeptide comprises all of nucleotides 1 to 1900 of the 5' coding sequence. Those skilled in the art will understand that according to the foregoing embodiments, the functional
5 fragment of the CATERPILLER 16.2 polypeptide will comprise the corresponding amino acids. In other embodiments, the nucleic acid encoding the functional fragment of CATERPILLER 16.2 comprises nucleotides 509-607 and/or nucleotides 2468 – 3489. In other particular embodiments, the functional fragment of CATERPILLER 16.2 comprises the C-terminus, e.g.,
10 from amino acids 728 to 1065 (see, **SEQ ID NO:28**). In still other representative embodiments, the functional CATERPILLER 16.2 fragment comprises at least about 20, 30, 50, 100 or 150 contiguous amino acids from the C-terminus from about amino acids 728 to 1065.

Likewise, those skilled in the art will appreciate that the present
15 invention also encompasses fusion proteins (and nucleic acid sequences encoding the same) comprising the CATERPILLER polypeptides of the invention (or a functional fragment thereof). For example, it may be useful to express the CATERPILLER polypeptide (or functional fragment) as a fusion protein that can be recognized by a commercially available antibody (e.g.,
20 FLAG motifs) or as a fusion protein that can otherwise be more easily purified (e.g., by addition of a poly-His tail). Additionally, fusion proteins that enhance the stability of the CATERPILLER polypeptide may be produced, e.g., fusion proteins comprising maltose binding protein (MBP) or glutathione-S-transferase. As another alternative, the fusion protein can comprise a
25 reporter molecule.

Likewise, it will be understood that the CATERPILLER polypeptides specifically disclosed herein will typically tolerate substitutions in the amino acid sequence and substantially retain biological activity. To identify polypeptides of the invention other than those specifically disclosed herein,
30 amino acid substitutions may be based on any characteristic known in the art, including the relative similarity or differences of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like.

Amino acid substitutions other than those disclosed herein may be achieved by changing the codons of the DNA sequence (or RNA sequence), according to the following codon table:

5

TABLE 2

Amino Acids			Codons						
Alanine	Ala	A	GCA	GCC	GCG	GCT			
Cysteine	Cys	C	TGC	TGT					
10 Aspartic acid	Asp	D	GAC	GAT					
Glutamic acid	Glu	E	GAA	GAG					
Phenylalanine	Phe	F	TTC	TTT					
Glycine	Gly	G	GGA	GGC	GGG	GGT			
Histidine	His	H	CAC	CAT					
15 Isoleucine	Ile	I	ATA	ATC	ATT				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	TTA	TTG	CTA	CTC	CTG	CTT	
Methionine	Met	M	ATG						
Asparagine	Asn	N	AAC	AAT					
20 Proline	Pro	P	CCA	CCC	CCG	CCT			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGT	
Serine	Ser	S	AGC	ACT	TCA	TCC	TCG	TCT	
Threonine	Thr	T	ACA	ACC	ACG	ACT			
25 Valine	Val	V	GTA	GTC	GTG	GTT			
Tryptophan	Trp	W	TGG						
Tyrosine	Tyr	Y	TAC	TAT					

30

In identifying amino acid sequences encoding CATERPILLER polypeptides other than those specifically disclosed herein, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is

generally understood in the art (see, Kyte and Doolittle, (1982) *J. Mol. Biol.* 157:105; incorporated herein by reference in its entirety). It is accepted that the relative hydrophobic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydrophobic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, *Id.*), these are:

isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

Accordingly, the hydrophobic index of the amino acid (or amino acid sequence) may be considered when modifying the CATERPILLER polypeptides specifically disclosed herein.

It is also understood in the art that the substitution of amino acids can be made on the basis of hydrophilicity. U.S. Patent No. 4,554,101

(incorporated herein by reference in its entirety) states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (± 3.0); aspartate ($+3.0 \pm 1$); glutamate ($+3.0 \pm 1$); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

Thus, the hydrophilicity of the amino acid (or amino acid sequence) may be considered when identifying additional CATERPILLER polypeptides beyond those specifically disclosed herein.

In embodiments of the invention, the nucleic acid encoding the CATERPILLER polypeptide (or functional fragment) will hybridize to the

nucleic acid sequences specifically disclosed herein or fragments thereof (e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:33, SEQ ID NO:148) under standard conditions as known by those skilled in the art and encode a functional CATERPILLER polypeptide or functional fragment thereof.

For example, hybridization of such sequences may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions (e.g., conditions represented by a wash stringency of 35-40% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 37°C; conditions represented by a wash stringency of 40-45% Formamide with 5x Denhardt's solution, 0.5% SDS, and 1x SSPE at 42°C; and conditions represented by a wash stringency of 50% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 42°C, respectively) to the nucleic acid sequences encoding the CATERPILLER polypeptides or functional fragments thereof specifically disclosed herein. See, e.g., Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2d Ed. 1989) (Cold Spring Harbor Laboratory).

In other embodiments, nucleic acid sequences encoding the CATERPILLER polypeptides of the invention have at least about 60%, 70%, 80%, 85%, 90%, 95%, 97% or higher sequence identity with the nucleic acid sequences specifically disclosed herein (or functional fragments thereof, as described above) and encode a functional CATERPILLER polypeptide or functional fragment thereof.

Further, it will be appreciated by those skilled in the art that there can be variability in the polynucleotides that encode the CATERPILLER polypeptides (and fragments thereof) of the present invention due to the degeneracy of the genetic code. The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same polypeptide, is well known in the literature (See, e.g., Table 2).

Likewise, the CATERPILLER polypeptides (and fragments thereof) of the invention include polypeptides that have at least about 60%, 70%, 80%, 85%, 90%, 95%, 97% or higher amino acid sequence identity with the polypeptide sequences specifically disclosed herein or fragments thereof (as

described above).

As is known in the art, a number of different programs can be used to identify whether a nucleic acid or polypeptide has sequence identity or similarity to a known sequence. Sequence identity or similarity may be determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman, *Adv. Appl. Math.* **2**, 482 (1981), by the sequence identity alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* **48**, 443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* **85**, 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux *et al.*, *Nucl. Acid Res.* **12**, 387-395 (1984), preferably using the default settings, or by inspection.

An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* **35**, 351-360 (1987); the method is similar to that described by Higgins & Sharp, *CABIOS* **5**, 151-153 (1989).

Another example of a useful algorithm is the BLAST algorithm, described in Altschul *et al.*, *J. Mol. Biol.* **215**, 403-410, (1990) and Karlin *et al.*, *Proc. Natl. Acad. Sci. USA* **90**, 5873-5787 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul *et al.*, *Methods in Enzymology*, **266**, 460-480 (1996); [http://blast.wustl.edu/blast/ README.html](http://blast.wustl.edu/blast/README.html). WU-BLAST-2 uses several search parameters, which are preferably set to the default values. The parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

An additional useful algorithm is gapped BLAST as reported by Altschul *et al. Nucleic Acids Res.* **25**, 3389-3402.

5 A percentage amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the "longer" sequence in the aligned region. The "longer" sequence is the one having the most actual residues in the aligned region (gaps introduced by WU-Blast-2 to maximize the alignment score are ignored).

10 In a similar manner, percent nucleic acid sequence identity with respect to the coding sequence of the polypeptides disclosed herein is defined as the percentage of nucleotide residues in the candidate sequence that are identical with the nucleotides in the polypeptide specifically disclosed herein.

The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer amino acids than the polypeptides specifically disclosed herein, it is
15 understood that in one embodiment, the percentage of sequence identity will be determined based on the number of identical amino acids in relation to the total number of amino acids. Thus, for example, sequence identity of sequences shorter than a sequence specifically disclosed herein, will be determined using the number of amino acids in the shorter sequence, in one
20 embodiment. In percent identity calculations relative weight is not assigned to various manifestations of sequence variation, such as, insertions, deletions, substitutions, *etc.*

In one embodiment, only identities are scored positively (+1) and all forms of sequence variation including gaps are assigned a value of "0", which
25 obviates the need for a weighted scale or parameters as described below for sequence similarity calculations. Percent sequence identity can be calculated, for example, by dividing the number of matching identical residues by the total number of residues of the "shorter" sequence in the aligned region and multiplying by 100. The "longer" sequence is the one having the most
30 actual residues in the aligned region.

Those skilled in the art will appreciate that the isolated nucleic acids encoding the CATERPILLER polypeptides of the invention will typically be

associated with appropriate expression control sequences, e.g., transcription/translation control signals and polyadenylation signals.

It will further be appreciated that a variety of promoter/enhancer elements can be used depending on the level and tissue-specific expression desired. The promoter can be constitutive or inducible, depending on the pattern of expression desired. The promoter can be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced. The promoter is chosen so that it will function in the target cell(s) of interest.

To illustrate, the CATERPILLER coding sequence can be operatively associated with a cytomegalovirus (CMV) major immediate-early promoter, an albumin promoter, an Elongation Factor 1- α (EF1- α) promoter, a PyK promoter, a MFG promoter, or a Rous sarcoma virus promoter.

Inducible promoter/enhancer elements include hormone-inducible and metal-inducible elements, and other promoters regulated by exogenously supplied compounds, including without limitation, the zinc-inducible metallothionein (MT) promoter; the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter; the T7 polymerase promoter system (see WO 98/10088); the ecdysone insect promoter (No et al, (1996) *Proc. Natl. Acad. Sci. USA* 93:3346); the tetracycline-repressible system (Gossen et al., (1992) *Proc. Natl. Acad. Sci. USA* 89:5547); the tetracycline-inducible system (Gossen et al., (1995) *Science* 268:1766; see also Harvey et al., (1998) *Curr. Opin. Chem. Biol.* 2:512); the RU486-inducible system (Wang et al., (1997) *Nat. Biotech.* 15:239; Wang et al., (1997) *Gene Ther.*, 4:432); and the rapamycin-inducible system (Magari et al., (1997) *J. Clin. Invest.* 100:2865).

Other tissue-specific promoters or regulatory promoters include, but are not limited to, promoters that typically confer tissue-specificity in myeloid-monocytic cells or cells of such origin (e.g., granulocytes, macrophages, monocytes, eosinophils, basophils, mast cells, dendritic cells, microglial, Langerhans cells), T cells, and B cells. These include but are not limited to promoters for GM-CSF, CD14, TCR, Ick, B220 and Ig.

Moreover, specific initiation signals are generally required for efficient translation of inserted protein coding sequences. These translational control sequences, which can include the ATG initiation codon and adjacent sequences, can be of a variety of origins, both natural and synthetic.

5 The present invention further provides cells comprising the isolated nucleic acids and polypeptides of the invention. The cell may be a cultured cell or a cell *in vivo*, *e.g.*, for use in therapeutic methods, screening methods, methods for studying the biological action of the CATERPILLER polypeptides, in methods of producing CATERPILLER polypeptides, or in methods of
10 maintaining or amplifying the nucleic acids of the invention, *etc.*

In particular embodiments, the cell is an untransformed cell or a cell from a cell line representing myeloid-monocytic cells or cells of such origin (*e.g.*, granulocytes, macrophages, monocytes, eosinophils, basophils, mast cells, dendritic cells, microglial, Langerhans cells). In other representative
15 embodiments, the cell is a T cell, B cell, epithelial cell, endothelial cell, or muscle cell.

The isolated nucleic acid can be incorporated into an expression vector. Expression vectors compatible with various host cells are well known in the art and contain suitable elements for transcription and translation of
20 nucleic acids. Typically, an expression vector contains an "expression cassette," which includes, in the 5' to 3' direction, a promoter, a coding sequence encoding a CATERPILLER polypeptide or functional fragment thereof operatively associated with the promoter, and, optionally, a termination sequence including a stop signal for RNA polymerase and a
25 polyadenylation signal for polyadenylase.

Expression vectors can be designed for expression of polypeptides in prokaryotic or eukaryotic cells. For example, polypeptides can be expressed in bacterial cells such as *E. coli*, insect cells (*e.g.*, the baculovirus expression system), yeast cells, plant cells or mammalian cells. Some suitable host cells
30 are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Examples of vectors for expression in the yeast *S. cerevisiae* include pYepSecI (Baldari et al., (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell*

30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, Calif.). Baculovirus vectors available for expression of nucleic acids to produce proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al., (1983) *Mol. Cell. Biol.* 3:2156-2165) and the pVL series (Lucklow, V.A., and Summers, M.d. (1989) *Virology* 170:31-39).

Examples of mammalian expression vectors include pCDM8 (Seed, (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987), *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus and Simian Virus 40.

In addition to the regulatory control sequences discussed above, the recombinant expression vector can contain additional nucleotide sequences. For example, the recombinant expression vector can encode a selectable marker gene to identify host cells that have incorporated the vector.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" refer to a variety of art-recognized techniques for introducing foreign nucleic acids (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, microinjection, DNA-loaded liposomes, lipofectamine-DNA complexes, cell sonication, gene bombardment using high velocity microprojectiles, and viral-mediated transfection. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory manuals.

If stable integration is desired, often only a small fraction of cells (in particular, mammalian cells) integrate the foreign DNA into their genome. In order to identify and select integrants, a nucleic acid that encodes a selectable marker (e.g., resistance to antibiotics) can be introduced into the host cells along with the nucleic acid of interest. Preferred selectable markers

include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acids encoding a selectable marker can be introduced into a host cell on the same vector as that comprising the nucleic acid of interest or can be introduced on a separate vector. Cells stably transfected
5 with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

The nucleic acid can also be introduced into a plant, plant cell or protoplast and, optionally, the isolated nucleic acid encoding the polypeptide
10 is integrated into the nuclear or plastidic genome. Plant transformation is known as the art. See, in general, *Methods in Enzymology* Vol. 153 ("Recombinant DNA Part D") 1987, Wu and Grossman Eds., Academic Press and European Patent Application EP 693554.

Foreign nucleic acids can be introduced into plant cells or protoplasts
15 by several methods. For example, nucleic acid can be mechanically transferred by microinjection directly into plant cells by use of micropipettes. Foreign nucleic acid can also be transferred into a plant cell by using polyethylene glycol which forms a precipitation complex with the genetic material that is taken up by the cell (Paszkowski et al. (1984) *EMBO J.*
20 3:2712-22). Foreign nucleic acid can be introduced into a plant cell by electroporation (Fromm et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:5824). In this technique, plant protoplasts are electroporated in the presence of plasmids or nucleic acids containing the relevant genetic construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing
25 the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form a plant callus. Selection of the transformed plant cells comprising the foreign nucleic acid can be accomplished using phenotypic markers.

Cauliflower mosaic virus (CaMV) can be used as a vector for
30 introducing foreign nucleic acids into plant cells (Hohn et al. (1982) "Molecular Biology of Plant Tumors," Academic Press, New York, pp. 549-560; Howell, U.S. Pat. No. 4,407,956). The CaMV viral DNA genome is inserted into a parent bacterial plasmid creating a recombinant DNA molecule which can be

propagated in bacteria. The recombinant plasmid can be further modified by introduction of the desired DNA sequence. The modified viral portion of the recombinant plasmid is then excised from the parent bacterial plasmid, and used to inoculate the plant cells or plants.

5 High velocity ballistic penetration by small particles can be used to introduce foreign nucleic acid into plant cells. Nucleic acid is disposed within the matrix of small beads or particles, or on the surface (Klein et al. (1987) *Nature* 327:70-73). Although typically only a single introduction of a new nucleic acid segment is required, this method also provides for multiple
10 introductions.

 A nucleic acid can be introduced into a plant cell by infection of a plant cell, an explant, a meristem or a seed with *Agrobacterium tumefaciens* transformed with the nucleic acid. Under appropriate conditions, the transformed plant cells are grown to form shoots, roots, and develop further
15 into plants. The nucleic acids can be introduced into plant cells, for example, by means of the Ti plasmid of *Agrobacterium tumefaciens*. The Ti plasmid is transmitted to plant cells upon infection by *Agrobacterium tumefaciens*, and is stably integrated into the plant genome (Horsch et al. (1984) "Inheritance of Functional Foreign Genes in Plants," *Science* 233:496498; Fraley et al. (1983)
20 *Proc. Natl. Acad. Sci. USA* 80:4803).

 Plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed so that whole plants are recovered which contain the transferred foreign nucleic acid. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*,
25 *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*,
30 *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

 Plant regeneration from cultured protoplasts is described in Evans et al., "Protoplasts Isolation and Culture," *Handbook of Plant Cell Cultures*

1:124-176 (MacMillan Publishing Co. New York 1983); M. R. Davey, "Recent Developments in the Culture and Regeneration of Plant Protoplasts," Protoplasts (1983)-Lecture Proceedings, pp. 12-29, (Birkhauser, Basel 1983); P. J. Dale, "Protoplast Culture and Plant Regeneration of Cereals and Other
5 Recalcitrant Crops," Protoplasts (1983)-Lecture Proceedings, pp. 31-41, (Birkhauser, Basel 1983); and H. Binding, "Regeneration of Plants," Plant Protoplasts, pp. 21-73, (CRC Press, Boca Raton 1985).

Regeneration from protoplasts varies from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the
10 exogenous sequence is first generated. In certain species, embryo formation can then be induced from the protoplast suspension, to the stage of ripening and germination as natural embryos. The culture medium, can contain various amino acids and hormones, such as auxin and cytokinins. It can also be advantageous to add glutamic acid and proline to the medium, especially
15 for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

Methods for generation of genetically engineered plants are further
20 described in U.S. Pat. Nos. 5,283,184, 5,482,852, and European Patent Application EP 693 554, all of which are hereby incorporated by reference.

As yet a further embodiment, the invention provides antibodies and antibody fragments that specifically bind to Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1 and/or
25 CATERPILLER 16.2.

The term "antibody" or "antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. The antibody can be monoclonal or polyclonal and can be of any species of origin, including (for example) mouse, rat, rabbit, horse, goat, sheep or human, or can be a
30 chimeric antibody. See, e.g., Walker et al., *Molec. Immunol.* 26, 403-11 (1989). The antibodies can be recombinant monoclonal antibodies produced according to the methods disclosed in U.S. Patent No. 4,474,893 or U.S.

Patent No. 4,816,567. The antibodies can also be chemically constructed according to the method disclosed in U.S. Patent No. 4,676,980.

Antibody fragments included within the scope of the present invention include, for example, Fab, F(ab')₂, and Fc fragments, and the corresponding
5 fragments obtained from antibodies other than IgG. Such fragments can be produced by known techniques. For example, F(ab')₂ fragments can be produced by pepsin digestion of the antibody molecule, and Fab fragments can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed to allow rapid and
10 easy identification of monoclonal Fab fragments with the desired specificity (Huse *et al.*, (1989) *Science* **254**, 1275-1281).

Polyclonal antibodies used to carry out the present invention can be produced by immunizing a suitable animal (e.g., rabbit, goat, etc.) with an antigen to which a monoclonal antibody to the target binds, collecting immune
15 serum from the animal, and separating the polyclonal antibodies from the immune serum, in accordance with known procedures. The inventors have successfully produced a polyclonal antibody to Monarch-1, which recognizes the protein in ELISA and western blot analysis. The inventors have also produced a polyclonal antibody to CIAS1.

20 Monoclonal antibodies used to carry out the present invention can be produced in a hybridoma cell line according to the technique of Kohler and Milstein, (1975) *Nature* **265**, 495-97. For example, a solution containing the appropriate antigen can be injected into a mouse and, after a sufficient time, the mouse sacrificed and spleen cells obtained. The spleen cells are then
25 immortalized by fusing them with myeloma cells or with lymphoma cells, typically in the presence of polyethylene glycol, to produce hybridoma cells. The hybridoma cells are then grown in a suitable medium and the supernatant screened for monoclonal antibodies having the desired specificity. Monoclonal Fab fragments can be produced in *E. coli* by recombinant
30 techniques known to those skilled in the art. See, e.g., W. Huse, (1989) *Science* **246**, 1275-81.

Antibodies specific to the target polypeptide can also be obtained by phage display techniques known in the art.

Various immunoassays can be used for screening to identify antibodies having the desired specificity for the polypeptides of this invention. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificity are well known in the art. Such immunoassays typically involve the measurement of complex formation between an antigen and its specific antibody (e.g., antigen/antibody complex formation). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the proteins or peptides of this invention can be used as well as a competitive binding assay.

Antibodies can be conjugated to a solid support (e.g., beads, plates, slides or wells formed from materials such as latex or polystyrene) in accordance with known techniques. Antibodies can likewise be conjugated to detectable groups such as radiolabels (e.g., ^{35}S , ^{125}I , ^{131}I), enzyme labels (e.g., horseradish peroxidase, alkaline phosphatase), and fluorescence labels (e.g., fluorescein) in accordance with known techniques. Determination of the formation of an antibody/antigen complex in the methods of this invention can be by detection of, for example, precipitation, agglutination, flocculation, radioactivity, color development or change, fluorescence, luminescence, etc., as is well known in the art.

III. Applications of the Present Invention.

CATERPILLER nucleic acids, polypeptides, antibodies, cells and other reagents have a wide variety of uses, both *in vitro* and *in vivo*. For example, in representative embodiments, these reagents may be used *in vitro* or *in vivo* (e.g., in an animal model) to study inflammatory pathways and apoptosis. Further, "knock in" and "knock out" animals can be used as animal models of disease or as screening tools (discussed more below) for compounds that interact with the CATERPILLER genes or polypeptides.

The invention can also be used to achieve therapeutic effects. The CATERPILLER nucleic acids and polypeptides are implicated in the regulation of diseases that are immediately inflammatory in nature, such as viral/bacterial/fungal/parasitic infections, sepsis, arthritis, type I diabetes,

allergies, hypersensitivity, systemic lupus, inflammatory bowel diseases (e.g., Crohn's disease), as well as diseases with strong inflammatory components, such as heart diseases, fibrosis, cancer, multiple sclerosis and other CNS disorders with an inflammatory component including Alzheimer's disease,

5 Parkinson's disease, and Huntington's disease. They can also play a role in clinically-induced conditions such as surgery and transplantation. According to the present invention, the activity of one or more CATERPILLER

polypeptides can be modulated (e.g., increased or decreased) to treat the above-mentioned inflammatory conditions. The activity of CATERPILLER

10 polypeptides can be directly regulated at the nucleic acid or protein level.

Alternatively, or additionally, the activity of CATERPILLER polypeptides can be indirectly modulated by regulating factors that are upstream or downstream in pathways involved in CATERPILLER activity or by regulating any other factor which results in modulation of CATERPILLER activity. Further,

15 interaction domains of CATERPILLER polypeptides with other polypeptides can be used to alter the function of either CATERPILLER or its interaction partner. As an illustration, the Monarch-1 polypeptide interacts with TRAF6 among several other proteins. This interaction site can be defined and used to identify small molecules that can mimic this interaction or block this

20 interaction. In addition, all members of the CATERPILLER family contain a nucleotide-binding domain. Nucleotide analogs may be used to modulate the function of this family. Many nucleotide-binding domains are associated with kinase activity, and such enzyme active sites are ideal targets for drug discovery.

25 Thus, in representative embodiments, the invention can be practiced to treat inflammatory conditions (including auto-inflammatory conditions) by modulating the activity of one or more CATERPILLER polypeptides.

Inflammatory conditions that can be treated according to the present invention include but are not limited to infections, sepsis, arthritis, type I diabetes,

30 allergies, hypersensitivity, systemic lupus, heart diseases, multiple sclerosis, asthma, fibrosis and inflammatory bowel diseases (e.g., Crohn's disease).

Further, CATERPILLER nucleic acids and polypeptides of the invention are involved in the Toll-like receptor (TLR) pathway (which is a group of

pattern recognition receptors for bacteria, viruses, fungus, protozoa, parasites and other pathogens). CATERPILLER family members can interfere with some of the TLR signaling molecules. Conversely, CATERPILLER polypeptides are also important for the synthesis of certain cytokines (IL-6, IL-10, IL-1) in response to TLR activation. Thus, the activity of one or more CATERPILLER polypeptides can be modulated to alter TLR pathway activity and/or to alter the response to pathogens, e.g., to decrease the inflammatory response to pathogens.

Moreover, in other embodiments, the CATERPILLER nucleic acids and polypeptides of the invention are involved in cell survival and apoptosis and are thus implicated in the control of abnormal cell proliferation (e.g., cancer and hyperplasia). This is supported by the effect of CATERPILLER polypeptides on NF- κ B and AP-1 function. In particular, NF- κ B is frequently considered a pro-survival signal, although the opposite effect has been reported as well. Similarly, AP-1 has a dual role in cell death and survival depending on the context. It has increasingly been recognized that apoptosis is a normal process that occurs during phagocytosis, embryonic differentiation, and organ development such as thymic development or brain development. Pathologic changes in apoptosis can lead to hyperplasia and cancer. Thus, the activity of one or more CATERPILLER polypeptides can be modulated to regulate cell survival and/or cell proliferation (e.g., to reduce abnormal cell proliferation and/or to treat cancer).

As used herein, the term "cancer" encompasses tumor-forming cancers. Likewise, the term "cancerous tissue" encompasses tumors.

The term "cancer" has its understood meaning in the art, for example, an uncontrolled growth of tissue that has the potential to spread to distant sites of the body (*i.e.*, metastasize). Exemplary cancers include, but are not limited to, leukemias, lymphomas, colon cancer, renal cancer, liver cancer, breast cancer, lung cancer, prostate cancer, cervical cancer, uterine cancer, ovarian cancer, melanoma, and the like. In embodiments of the invention, the cancer is a brain cancer or other cancer of the CNS.

The term "tumor" is also understood in the art, for example, as an abnormal mass of undifferentiated cells within a multicellular organism. Tumors can be malignant or benign.

By the terms "treating cancer" or "treatment of cancer", it is intended that the severity of the cancer is reduced or the cancer is at least partially eliminated. In particular embodiments, these terms indicate that metastasis of the cancer is reduced or at least partially eliminated. By the terms "prevention of cancer" or "preventing cancer" it is intended that the methods at least partially eliminate or reduce the incidence or onset of cancer. Alternatively stated, the onset of cancer in the subject may be slowed, controlled, decreased in likelihood or probability, or delayed.

It is known in the art that immune responses may be enhanced by immunomodulatory cytokines (e.g., α -interferon, β -interferon, γ -interferon, ω -interferon, τ -interferon, interleukin-1 α , interleukin-1 β , interleukin-2, interleukin-3, interleukin-4, interleukin 5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin 12, interleukin-13, interleukin-14, interleukin-18, B cell Growth factor, CD40 Ligand, tumor necrosis factor- α , tumor necrosis factor- β , monocyte chemoattractant protein-1, granulocyte-macrophage colony stimulating factor, and lymphotoxin).

Accordingly, immunomodulatory cytokines (preferably, CTL inductive cytokines) may be co-administered to a subject.

Cytokines may be administered by any method known in the art. Exogenous cytokines may be administered to the subject, or alternatively, a nucleotide sequence encoding a cytokine may be delivered to the subject using a suitable vector, and the cytokine produced *in vivo*.

As one particular representative example of how the present invention may be used, Monarch-1 is an immune regulatory factor that upregulates classical and nonclassical class I MHC and its processing molecules (LMP7). It also upregulates a TNF family member, and an IFN-induced DNA-binding protein. Compounds that modulate the expression or function of Monarch-1 can be used to influence these downstream targets. To illustrate, the modulation of MHC is important for transplantation where a downregulation of MHC is preferred. Down-regulation of MHC can also be advantageous for

controlling auto-inflammatory and immune conditions. Further, Monarch-1 causes the induction of IL-10, IL-6 and IL-1 in that the elimination of Monarch-1 enhances the level of these cytokines. Enhancement of MHC can be beneficial for combating infections, such as viral, bacterial, protozoan, yeast or fungal infections. Interfering with Monarch-1 can decrease inflammatory response to bacterial and other pathogens.

Furthermore, the inventors have found that Monarch-1 induces the expression of IFI16, a type I and type II interferon induced DNA-binding factor that is known to regulate genes through binding to NF- κ B or to downregulate the function of another DNA-binding protein, AP-1. Monarch-1 downregulates AP-1 function, and it also decreases AP-1 binding to DNA. Downregulation of AP-1 is known to modulate inflammatory, growth and differentiation pathways. Interfering with Monarch-1 can be used to interfere with AP-1 function.

In addition, Monarch-1 causes the induction of TNF ligand or CD70. CD70 is known to enhance CD8 T cell responses. Hence, modulation of Monarch-1 can alter T cell responses.

Overall, Monarch-1 is involved in the regulation of cell survival (due to effects on NF- κ B and AP-1) and inflammation. Monarch-1 activity can be modulated to treat auto-inflammatory and inflammatory conditions and/or uncontrolled cell growth (cancer, hyperplasia).

As a further illustrative embodiment, it has further been found that another CATERPILLER gene, CIAS1, is activated by all bacterial and viral products evaluated, including LPS, lipoteichoic acid, proteoglycans, and double-stranded RNA. CIAS1 downregulates the NF- κ B response and the activation of MHC class II molecules. These two responses are important in innate and adaptive immunity. CIAS1 is increased in a number of models of inflammation. CIAS1 also modulates NF- κ B function by preventing its translocation into the nucleus. Domain analysis indicates the importance of the NBD-LRR region in this function. Thus, modulation of CIAS1 expression or function can alter responses to pathogens and can be important in treating sepsis, bacteremia, anti-viral responses, and inflammatory conditions (including auto-inflammatory conditions), in the treatment of uncontrolled cell growth (cancer, hyperplasia) and/or reducing transplant rejection.

Likewise, CATERPILLER 11.2, CATERPILLER 11.3 and CATERPILLER 16.2 reduce the function of NF- κ B, which is involved in both inflammatory responses and cell survival. Thus, modulation of NF- κ B activity can be used to regulate immunity and cell survival as NF- κ B controls these processes. In addition, CATERPILLER 11.2 suppresses the expression of the MHC-II promoter. Proper MHC-II expression is involved in immune recognition to elicit T cell responses against pathogens (viral, bacterial, fungal and parasitic) and antigens and is also implicated in transplant rejection and in autoimmune diseases. CATERPILLER 16.2 expression is reduced by bacterial products that activate the Toll-like receptor pathway, suggesting that CATERPILLER 16.2 is part of the Toll-like receptor pathway. CATERPILLER 11.3 also reduces the function of TLR activated signaling molecules, such as MyD88, and is found to be expressed at high levels in the T regulatory cells. T regulatory cells are typically associated with the suppression of adaptive T cell responses, and are now targets of cancer treatment (e.g., removal of T regulatory cells to enhance anti-cancer immunity), transplantation (e.g., enhancement of T regulatory cells to improve graft acceptance) and other immune responses. CATERPILLER 11.2, CATERPILLER 11.3 and/or CATERPILLER 16.2 activity can be modulated to treat a variety of autoimmune inflammatory or inflammatory conditions (as described above), in the containment of transplant rejection, regulation of anti-pathogen responses, and/or in the treatment of uncontrolled cell growth (cancer, hyperplasia). Modulation of CATERPILLER polypeptide or nucleic acid activity can also be used to inhibit NF- κ B or NF- κ B-dependent pathways, which may be pro-survival or pro-apoptotic as well as pro- or anti-inflammatory depending on the context. For example, NF- κ B is found to be important in many pro-inflammatory conditions; however, more recent evidence also suggest that it is important in the reparation phase after the initial inflammatory phase.

According to the foregoing methods, one or more CATERPILLER polypeptides (or functional fragment thereof) can be introduced into a cell or administered to a subject. Alternatively, a nucleic acid encoding the polypeptide(s) (or functional fragment) can be delivered so that the polypeptide(s) is produced in the cell or subject. As described in more detail

hereinbelow, these polypeptides (or fragments thereof) can be used to screen for small molecules that can interact with them to enhance or block their function. As an example, the nucleotide binding domain is an ideal target that can be associated with kinase activity, and enzyme sites are particularly suited as drug target sites.

It will be apparent to those skilled in the art that any suitable vector can be used to deliver the nucleic acid to a cell or subject. The choice of delivery vector can be made based on a number of factors known in the art, including age and species of the target host, *in vitro* vs. *in vivo* delivery, level and persistence of expression desired, intended purpose (e.g., for therapy or screening), the target cell or organ, route of delivery, size of the isolated nucleic acid, safety concerns, and the like.

Suitable vectors include virus vectors (e.g., retrovirus, alphavirus; vaccinia virus; adenovirus, adeno-associated virus and other parvoviruses, retrovirus, lentivirus poxvirus or herpes simplex virus), lipid-lysine vectors, synthetic polyamino polymer vectors, and the like.

Any viral vector that is known in the art can be used in the present invention. Examples of such viral vectors include, but are not limited to vectors derived from: Adenoviridae; Birnaviridae; Bunyaviridae; Caliciviridae, Capillovirus group; Carlavirus group; Carmovirus virus group; Group Caulimovirus; Closterovirus Group; Commelina yellow mottle virus group; Comovirus virus group; Coronaviridae; PM2 phage group; Corbicoviridae; Group Cryptic virus; group Cryptovirus; Cucumovirus virus group Family ([PHgr]6 phage group; Cysioviridae; Group Carnation ringspot; Dianthovirus virus group; Group Broad bean wilt; Fabavirus virus group; Filoviridae; Flaviviridae; Furovirus group; Group Germinivirus; Group Giardiavirus; Hepadnaviridae; Herpesviridae; Hordeivirus virus group; Illarvirus virus group; Inoviridae; Iridoviridae; Leviviridae; Lipothrixviridae; Luteovirus group; Marafivirus virus group; Maize chlorotic dwarf virus group; icroviridae; Myoviridae; Necrovirus group; Nepovirus virus group; Nodaviridae; Orthomyxoviridae; Papovaviridae; Paramyxoviridae; Parsnip yellow fleck virus group; Partitiviridae; Parvoviridae; Pea enation mosaic virus group; Phycodnaviridae; Picomaviridae; Plasmaviridae; Prodoviridae;

Polydnaviridae; Potexvirus group; Potyvirus; Poxviridae; Reoviridae; Retroviridae; Rhabdoviridae; Group Rhizidiovirus; Siphoviridae; Sobemovirus group; SSV 1-Type Phages; Tectiviridae; Tenuivirus; Tetraviridae; Group Tobamovirus; Group Tobravirus; Togaviridae; Group Tombusvirus; Group Torovirus; Totiviridae; Group Tymovirus; and Plant virus satellites.

Protocols for producing recombinant viral vectors and for using viral vectors for nucleic acid delivery can be found in *Current Protocols in Molecular Biology*, Ausubel, F. M. et al. (eds.) Greene Publishing Associates, (1989) and other standard laboratory manuals (e.g., *Vectors for Gene Therapy*. In: *Current Protocols in Human Genetics*. John Wiley and Sons, Inc.: 1997).

Non-viral transfer methods can also be employed. Many non-viral methods of nucleic acid transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In particular embodiments, non-viral nucleic acid delivery systems rely on endocytic pathways for the uptake of the nucleic acid molecule by the targeted cell. Exemplary nucleic acid delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

In particular embodiments, plasmid vectors are used in the practice of the present invention. For example, naked plasmids can be introduced into muscle cells by injection into the tissue. Expression can extend over many months, although the number of positive cells is typically low (Wolff et al., (1989) *Science* **247**:247). Cationic lipids have been demonstrated to aid in introduction of nucleic acids into some cells in culture (Felgner and Ringold, (1989) *Nature* **337**:387). Injection of cationic lipid plasmid DNA complexes into the circulation of mice has been shown to result in expression of the DNA in lung (Brigham et al., (1989) *Am. J. Med. Sci.* **298**:278). One advantage of plasmid DNA is that it can be introduced into non-replicating cells.

In a representative embodiment, a nucleic acid molecule (e.g., a plasmid) can be entrapped in a lipid particle bearing positive charges on its surface and, optionally, tagged with antibodies against cell surface antigens of the target tissue (Mizuno et al., (1992) *No Shinkei Geka* **20**:547; PCT

publication WO 91/06309; Japanese patent application 1047381; and European patent publication EP-A-43075).

Liposomes that consist of amphiphilic cationic molecules are useful as non-viral vectors for nucleic acid delivery *in vitro* and *in vivo* (reviewed in Crystal, *Science* **270**: 404-410 (1995); Blaese et al., *Cancer Gene Ther.* **2**: 291-297 (1995); Behr et al., *Bioconjugate Chem.* **5**: 382-389 (1994); Remy et al., *Bioconjugate Chem.* **5**: 647-654 (1994); and Gao et al., *Gene Therapy* **2**: 710-722 (1995)). The positively charged liposomes are believed to complex with negatively charged nucleic acids via electrostatic interactions to form lipid:nucleic acid complexes. The lipid:nucleic acid complexes have several advantages as nucleic acid transfer vectors. Unlike viral vectors, the lipid:nucleic acid complexes can be used to transfer expression cassettes of essentially unlimited size. Since the complexes lack proteins, they can evoke fewer immunogenic and inflammatory responses. Moreover, they cannot replicate or recombine to form an infectious agent and have low integration frequency. A number of publications have demonstrated that amphiphilic cationic lipids can mediate nucleic acid delivery *in vivo* and *in vitro* (Felgner et al., *Proc. Natl. Acad. Sci. USA* **84**: 7413-17 (1987); Loeffler et al., *Methods in Enzymology* **217**: 599-618 (1993); Felgner et al., *J. Biol. Chem.* **269**: 2550-2561 (1994)).

Several groups have reported the use of amphiphilic cationic lipid:nucleic acid complexes for *in vivo* transfection both in animals and in humans (reviewed in Gao et al., *Gene Therapy* **2**: 710-722 (1995); Zhu et al., *Science* **261**: 209-211 (1993); and Thierry et al., *Proc. Natl. Acad. Sci. USA* **92**: 9742-9746 (1995)). U.S. Patent No. 6,410,049 describes a method of preparing cationic lipid:nucleic acid complexes that have a prolonged shelf life.

In other embodiments, it is desired to reduce or inhibit the activity of one or more CATERPILLER polypeptides. The activity of CATERPILLER polypeptides can be inhibited at the nucleic acid or protein level. Alternatively, or additionally, the activity of CATERPILLER polypeptides can be indirectly inhibited by regulating factors that are upstream or downstream in pathways

involved in CATERPILLER activity or by regulating any other factor which results in inhibition of CATERPILLER activity.

Numerous methods for reducing the activity of one or more CATERPILLER polypeptides *in vitro* or *in vivo* are known. For example, the coding and noncoding nucleotide sequences for a number of CATERPILLER genes are disclosed herein or are otherwise known in the art. An antisense nucleotide sequence or nucleic acid encoding an antisense nucleotide sequence can be generated to any portion thereof in accordance with known techniques.

The term "antisense nucleotide sequence" or "antisense oligonucleotide" as used herein, refers to a nucleotide sequence that is complementary to a specified DNA or RNA sequence. Antisense oligonucleotides and nucleic acids that express the same can be made in accordance with conventional techniques. See, e.g., U.S. Patent No. 5,023,243 to Tullis; U.S. Patent No. 5,149,797 to Pederson et al.

As illustrative examples of an antisense nucleotide sequence that can be used to carry out the invention is a nucleotide sequence that is complementary to the nucleotide sequences of **SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:33 or SEQ ID NO:148** (or a portion thereof of at least 10, 20, 40, 50, 75, 100, 150, 200, 300, 500 or 1000 contiguous bases) and will reduce the level of polypeptide production.

Those skilled in the art will appreciate that it is not necessary that the antisense nucleotide sequence be fully complementary to the target sequence as long as the degree of sequence similarity is sufficient for the antisense nucleotide sequence to hybridize to its target and reduce production of the polypeptide. As is known in the art, a higher degree of sequence similarity is generally required for short antisense nucleotide sequences, whereas a greater degree of mismatched bases will be tolerated by longer antisense nucleotide sequences.

In representative embodiments of the invention, the antisense nucleotide sequence will hybridize to the nucleotide sequences encoding the CATERPILLER polypeptides specifically disclosed herein (e.g., **SEQ ID NO:1,**

SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:33 or SEQ ID NO:148 or portions thereof) and will reduce the level of polypeptide production.

5 For example, hybridization of such nucleotide sequences can be carried out under conditions of reduced stringency, medium stringency or even stringent conditions (e.g., conditions represented by a wash stringency of 35-40% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 37°C; conditions represented by a wash stringency of 40-45% Formamide
10 with 5x Denhardt's solution, 0.5% SDS, and 1x SSPE at 42°C; and/or conditions represented by a wash stringency of 50% Formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 42°C, respectively) to the nucleotide sequences specifically disclosed herein. See, e.g., Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2d Ed. 1989) (Cold Spring
15 Harbor Laboratory).

 In other embodiments, antisense nucleotide sequences of the invention have at least about 60%, 70%, 80%, 90%, 95%, 97%, 98% or higher sequence similarity with the complement of the coding sequences specifically disclosed herein and will reduce the level of polypeptide production.

20 In other embodiments, the antisense nucleotide sequence can be directed against any coding sequence, the silencing of which results in a modulation of a CATERPILLER polypeptide.

 The length of the antisense nucleotide sequence (i.e., the number of nucleotides therein) is not critical as long as it binds selectively to the intended
25 location and reduces transcription and/or translation of the target sequence, and can be determined in accordance with routine procedures. In general, the antisense nucleotide sequence will be from about eight, ten or twelve nucleotides in length up to about 20, 30, 50, 75 or 100 nucleotides, or longer, in length.

30 An antisense nucleotide sequence can be constructed using chemical synthesis and enzymatic ligation reactions by procedures known in the art. For example, an antisense nucleotide sequence can be chemically synthesized using naturally occurring nucleotides or various modified

nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleotide sequences, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleotide sequence include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleotide sequence can be produced using an expression vector into which a nucleic acid has been cloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest).

The antisense nucleotide sequences of the invention further include nucleotide sequences wherein at least one, or all, or the internucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphonothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates. For example, every other one of the internucleotide bridging phosphate residues can be modified as described. In another non-limiting example, the antisense nucleotide sequence is a nucleotide sequence in which one, or all, of the nucleotides contain a 2' loweralkyl moiety (e.g., C₁-C₄, linear or branched, saturated or unsaturated alkyl, such as methyl, ethyl, ethenyl, propyl, 1-propenyl, 2-

propenyl, and isopropyl). For example, every other one of the nucleotides can be modified as described. See also, Furdon et al., (1989) *Nucleic Acids Res.* **17**, 9193-9204; Agrawal et al., (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1401-1405; Baker et al., (1990) *Nucleic Acids Res.* **18**, 3537-3543; Sproat et al.,
5 (1989) *Nucleic Acids Res.* **17**, 3373-3386; Walder and Walder, (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5011-5015; incorporated by reference herein in their entireties for their teaching of methods of making antisense molecules, including those containing modified nucleotide bases).

Triple helix base-pairing methods can also be employed to inhibit
10 production of CATERPILLER polypeptides. Triple helix pairing is believed to work by inhibiting the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (e.g., Gee et al., (1994) In: Huber et al., *Molecular and Immunologic*
15 *Approaches*, Futura Publishing Co., Mt. Kisco, NY).

Small Interference (si) RNA, also known as RNA interference (RNAi) molecules, provides another approach for modulating CATERPILLER polypeptide activity. The siRNA can be directed against the CATERPILLER nucleic acid sequence or any other sequence that results in modulation of
20 CATERPILLER activity.

siRNA is a mechanism of post-transcriptional gene silencing in which double-stranded RNA (dsRNA) corresponding to a coding sequence of interest is introduced into a cell or an organism, resulting in degradation of the corresponding mRNA. The siRNA effect persists for multiple cell divisions
25 before gene expression is regained. siRNA is therefore a powerful method for making targeted knockouts or "knockdowns" at the RNA level. siRNA has proven successful in human cells, including human embryonic kidney and HeLa cells (see, e.g., Elbashir et al., *Nature* (2001) **411**:494-8). In one embodiment, silencing can be induced in mammalian cells by enforcing
30 endogenous expression of RNA hairpins (see Paddison et al., (2002), *PNAS USA* **99**:1443-1448). In another embodiment, transfection of small (21-23 nt) dsRNA specifically inhibits nucleic acid expression (reviewed in Caplen, (2002) *Trends in Biotechnology* **20**:49-51).

The mechanism by which siRNA achieves gene silencing has been reviewed in Sharp et al, (2001) *Genes Dev* **15**: 485-490; and Hammond et al., (2001) *Nature Rev Gen* **2**:110-119).

siRNA technology utilizes standard molecular biology methods.

- 5 dsRNA corresponding to all or a part of a target coding sequence to be inactivated can be produced by standard methods, e.g., by simultaneous transcription of both strands of a template DNA (corresponding to the target sequence) with T7 RNA polymerase. Kits for production of dsRNA for use in siRNA are available commercially, e.g., from New England Biolabs, Inc.
- 10 Methods of transfection of dsRNA or plasmids engineered to make dsRNA are routine in the art.

- Silencing effects similar to those produced by siRNA have been reported in mammalian cells with transfection of a mRNA-cDNA hybrid construct (Lin et al., (2001) *Biochem Biophys Res Commun* **281**:639-44),
- 15 providing yet another strategy for silencing a coding sequence of interest.

In particular embodiments, the siRNA molecules comprise **SEQ ID NO:122** and/or **SEQ ID NO:123** (Monarch-1), **SEQ ID NO:133** (CATERPILLER 11.2); **SEQ ID NO:134** (CATERPILLER 16.1) or **SEQ ID NO:144** and/or **SEQ ID NO:145** (CATERPILLER 16.2).

- 20 CATERPILLER polypeptide activity can also be inhibited using ribozymes. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim et al., (1987) *Proc. Natl. Acad. Sci. USA* **84**:8788; Gerlach et al., (1987) *Nature* **328**:802; Forster and Symons, (1987)
- 25 *Cell* **49**:211). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Michel and Westhof, (1990) *J. Mol. Biol.* **216**:585; Reinhold-Hurek and Shub, (1992) *Nature* **357**:173). This specificity has been attributed to the
- 30 requirement that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

Ribozyme catalysis has primarily been observed as part of sequence-specific cleavage/ligation reactions involving nucleic acids (Joyce, (1989)

Nature 338:217). For example, U.S. Pat. No. 5,354,855 reports that certain ribozymes can act as endonucleases with a sequence specificity greater than that of known ribonucleases and approaching that of the DNA restriction enzymes. Thus, sequence-specific ribozyme-mediated inhibition of gene expression may be particularly suited to therapeutic applications (Scanlon et al., (1991) *Proc. Natl. Acad. Sci. USA* 88:10591; Sarver et al., (1990) *Science* 247:1222; Sioud et al., (1992) *J. Mol. Biol.* 223:831).

CATERPILLER polypeptide activity can further be modulated by interaction with an antibody or antibody fragment. The antibody or antibody fragment can bind to the CATERPILLER polypeptide (e.g., at the nucleotide binding site) or to any other polypeptide of interest (e.g., TRAF6, for example, at the active site), as long as the binding between the antibody or the antibody fragment and the target polypeptide results in modulation of the CATERPILLER polypeptide activity. Antibodies and antibody fragments are as described in more detail hereinabove.

Furthermore, the present invention provides a method of modulating the activity of a CATERPILLER polypeptide (e.g., for therapy or other purposes described above), comprising administering to a cell or to a subject a compound that modulates the activity of a CATERPILLER polypeptide, the compound administered in an amount effective to modulate the activity of the CATERPILLER polypeptide. The compound can enhance or inhibit the activity of the CATERPILLER polypeptide. Further, the compound can interact directly with the CATERPILLER polypeptide (e.g., by binding to the nucleotide binding domain) or at the nucleic acid level to modulate the activity of the polypeptide. Alternatively, the compound can interact with any other polypeptide, nucleic acid or other molecule (e.g., a nucleotide analog that binds to the nucleotide binding domain) if such interaction results in a modulation of the activity of the CATERPILLER polypeptide.

For example, TRAF6 (an enzyme) associates with Monarch-1 and enhances the degradation of Monarch-1. A compound that interferes with this interaction between TRAF6 and Monarch-1 (e.g., by decreasing the production or activity of TRAF6 or by binding to one of the two polypeptides

and blocking TRAF6 binding to Monarch-1) can be used to enhance Monarch-1 activity.

The term "compound" as used herein is intended to be interpreted broadly and encompasses organic and inorganic molecules. Organic compounds include, but are not limited to polypeptides, lipids, carbohydrates, coenzymes and nucleic acid molecules (*e.g.*, gene delivery vectors, antisense oligonucleotides, siRNA, all as described above).

Polypeptides include but are not limited to antibodies (described in more detail above) and enzymes. Nucleic acids include but are not limited to DNA, RNA and DNA-RNA chimeric molecules. Suitable RNA molecules include siRNA, antisense RNA molecules and ribozymes (all of which are described in more detail above). The nucleic acid can further encode any polypeptide such that administration of the nucleic acid and production of the polypeptide results in a modulation of the activity of a CATERPILLER polypeptide.

The compound can further be a compound that is identified by any of the screening methods described below.

The compounds of the present invention can optionally be administered in conjunction with other therapeutic agents. The additional therapeutic agents can be administered concurrently with the compounds of the invention. As used herein, the word "concurrently" means sufficiently close in time to produce a combined effect (that is, concurrently can be simultaneously, or it can be two or more events occurring within a short time period before or after each other).

As a further aspect, the invention provides pharmaceutical formulations and methods of administering the same to achieve any of the therapeutic effects (*e.g.*, anti-inflammatory, inhibition of abnormal cell proliferation, *etc.*) discussed above. The pharmaceutical formulation may comprise any of the reagents discussed above in a pharmaceutically acceptable carrier, *e.g.*, a nucleic acid encoding a CATERPILLER polypeptide or a fragment thereof, a CATERPILLER polypeptide or fragment thereof, an antibody against a CATERPILLER polypeptide, an antisense oligonucleotide, an siRNA molecule, a ribozyme, or any other compound that modulates the activity of a CATERPILLER polypeptide including compounds identified by the screening methods described herein.

Small molecules or peptidomimetics that can bind to certain domains of a CATERPILLER polypeptide (e.g., pyrin, CARD, NBC, LRR, etc.) to enhance or block the function of the polypeptide is another pharmaceutical approach. If the polypeptide has an enzyme activity, as is frequently found for NBD sequences, molecules that can block the enzyme activity are well-suited as pharmaceutical compounds as they are exponential in efficiency due to the nature of enzyme reactions.

By "pharmaceutically acceptable" it is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject without causing any undesirable biological effects such as toxicity.

The formulations of the invention can optionally comprise medicinal agents, pharmaceutical agents, carriers, adjuvants, dispersing agents, diluents, and the like.

The compounds of the invention can be formulated for administration in a pharmaceutical carrier in accordance with known techniques. See, e.g., Remington, *The Science And Practice of Pharmacy* (9th Ed. 1995). In the manufacture of a pharmaceutical formulation according to the invention, the compound (including the physiologically acceptable salts thereof) is typically admixed with, *inter alia*, an acceptable carrier. The carrier can be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose formulation, for example, a tablet, which can contain from 0.01 or 0.5% to 95% or 99% by weight of the compound. One or more compounds can be incorporated in the formulations of the invention, which can be prepared by any of the well-known techniques of pharmacy.

A further aspect of the invention is a method of treating subjects *in vivo*, comprising administering to a subject a pharmaceutical composition comprising a compound of the invention in a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is administered in a therapeutically effective amount. Administration of the compounds of the present invention to a human subject or an animal in need thereof can be by any means known in the art for administering compounds.

The formulations of the invention include those suitable for oral, rectal, topical, buccal (e.g., sub-lingual), vaginal, parenteral (e.g., subcutaneous,

intramuscular including skeletal muscle, cardiac muscle, diaphragm muscle and smooth muscle, intradermal, intravenous, intraperitoneal), topical (*i.e.*, both skin and mucosal surfaces, including airway surfaces), intranasal, transdermal, intraarticular, intrathecal and inhalation administration,

5 administration to the liver by intraportal delivery, as well as direct organ injection (*e.g.*, into the liver, into the brain for delivery to the central nervous system, into the pancreas). The most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular compound which is being used.

10 For injection, the carrier will typically be a liquid, such as sterile pyrogen-free water, pyrogen-free phosphate-buffered saline solution, bacteriostatic water, or Cremophor EL[R] (BASF, Parsippany, N.J.). For other methods of administration, the carrier can be either solid or liquid.

For oral administration, the compound can be administered in solid
15 dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. Compounds can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium
20 saccharin, talcum, magnesium carbonate and the like. Examples of additional inactive ingredients that can be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, edible white ink and the like. Similar diluents can be used to make compressed tablets.

25 Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage
30 forms for oral administration can contain coloring and flavoring to increase patient acceptance.

Formulations suitable for buccal (sub-lingual) administration include lozenges comprising the compound in a flavored base, usually sucrose and

acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations of the present invention suitable for parenteral administration comprise sterile aqueous and non-aqueous injection solutions of the compound, which preparations are preferably isotonic with the blood of the intended recipient. These preparations can contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions can include suspending agents and thickening agents. The formulations can be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described. For example, in one aspect of the present invention, there is provided an injectable, stable, sterile composition comprising a compound of the invention, in a unit dosage form in a sealed container. The compound or salt is provided in the form of a lyophilizate which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection thereof into a subject. The unit dosage form typically comprises from about 10 mg to about 10 grams of the compound or salt. When the compound or salt is substantially water-insoluble, a sufficient amount of emulsifying agent which is pharmaceutically acceptable can be employed in sufficient quantity to emulsify the compound or salt in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These can be prepared by admixing the compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Formulations suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which can be used include petroleum jelly, lanoline, polyethylene

glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

Formulations suitable for transdermal administration can be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Formulations suitable for transdermal administration can also be delivered by iontophoresis (see, for example, *Pharmaceutical Research* 3 (6):318 (1986)) and typically take the form of an optionally buffered aqueous solution of the compound. Suitable formulations comprise citrate or bis/tris buffer (pH 6) or ethanol/water and contain from 0.1 to 0.2M of the compound.

The compound can alternatively be formulated for nasal administration or otherwise administered to the lungs of a subject by any suitable means, but is preferably administered by an aerosol suspension of respirable particles comprising the compound, which the subject inhales. The respirable particles can be liquid or solid. The term "aerosol" includes any gas-borne suspended phase, which is capable of being inhaled into the bronchioles or nasal passages. Specifically, aerosol includes a gas-borne suspension of droplets, as can be produced in a metered dose inhaler or nebulizer, or in a mist sprayer. Aerosol also includes a dry powder composition suspended in air or other carrier gas, which can be delivered by insufflation from an inhaler device, for example. See Ganderton & Jones, *Drug Delivery to the Respiratory Tract*, Ellis Horwood (1987); Gonda (1990) *Critical Reviews in Therapeutic Drug Carrier Systems* 6:273-313; and Raeburn et al. (1992) *J. Pharmacol. Toxicol. Methods* 27:143-159. Aerosols of liquid particles comprising the compound can be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. See, e.g., U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the compound can likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

Alternatively, one can administer the compound in a local rather than systemic manner, for example, in a depot or sustained-release formulation.

Further, the present invention provides liposomal formulations of the compounds disclosed herein and salts thereof. The technology for forming liposomal suspensions is well known in the art. When the compound or salt thereof is an aqueous-soluble salt, using conventional liposome technology, the same can be incorporated into lipid vesicles. In such an instance, due to the water solubility of the compound or salt, the compound or salt will be substantially entrained within the hydrophilic center or core of the liposomes. The lipid layer employed can be of any conventional composition and can either contain cholesterol or can be cholesterol-free. When the compound or salt of interest is water-insoluble, again employing conventional liposome formation technology, the salt can be substantially entrained within the hydrophobic lipid bilayer which forms the structure of the liposome. In either instance, the liposomes which are produced can be reduced in size, as through the use of standard sonication and homogenization techniques.

The liposomal formulations containing the compounds disclosed herein or salts thereof, can be lyophilized to produce a lyophilizate which can be reconstituted with a pharmaceutically acceptable carrier, such as water, to regenerate a liposomal suspension.

In the case of water-insoluble compounds, a pharmaceutical composition can be prepared containing the water-insoluble compound, such as for example, in an aqueous base emulsion. In such an instance, the composition will contain a sufficient amount of pharmaceutically acceptable emulsifying agent to emulsify the desired amount of the compound. Particularly useful emulsifying agents include phosphatidyl cholines and lecithin.

In particular embodiments, the compound is administered to the subject in a therapeutically effective amount, as that term is defined above. Dosages of pharmaceutically active compounds can be determined by methods known in the art, see, e.g., *Remington's Pharmaceutical Sciences* (Maack Publishing Co., Easton, Pa). The therapeutically effective dosage of any specific compound will vary somewhat from compound to compound, and patient to patient, and will depend upon the condition of the patient and the route of delivery. As a general proposition, a dosage from about 0.1 to about

50 mg/kg will have therapeutic efficacy, with all weights being calculated based upon the weight of the compound, including the cases where a salt is employed. Toxicity concerns at the higher level can restrict intravenous dosages to a lower level such as up to about 10 mg/kg, with all weights being
5 calculated based upon the weight of the compound, including the cases where a salt is employed. A dosage from about 10 mg/kg to about 50 mg/kg can be employed for oral administration. Typically, a dosage from about 0.5 mg/kg to 5 mg/kg can be employed for intramuscular injection. Particular dosages are about 1 μ mol/kg to 50 μ mol/kg, and more particularly to about 22
10 μ mol/kg and to 33 μ mol/kg of the compound for intravenous or oral administration, respectively.

In particular embodiments of the invention, more than one administration (e.g., two, three, four, or more administrations) can be employed over a variety of time intervals (e.g., hourly, daily, weekly, monthly,
15 etc.) to achieve therapeutic effects.

The present invention finds use in veterinary and medical applications. Suitable subjects include both avians and mammals, with mammals being preferred. The term "avian" as used herein includes, but is not limited to, chickens, ducks, geese, quail, turkeys and pheasants. The term "mammal" as
20 used herein includes, but is not limited to, humans, bovines, ovines, caprines, equines, felines, canines, lagomorphs, etc. Human subjects include neonates, infants, juveniles, and adults. In other embodiments, the subject is an animal model of inflammatory disease or cancer.

The CATERPILLER nucleic acids may further be used as
25 chromosomal markers, *i.e.*, to map the location of other genes. As another embodiment, the CATERPILLER nucleic acids can be used as genetic markers of diseases, *e.g.*, inflammatory and autoimmune diseases. For example, Monarch-1 maps to the multiple sclerosis susceptibility region and CATERPILLER 16.1 maps within the Crohn's disease susceptibility region.
30 Linkage of these genes with diseases will facilitate gene typing whereby certain allelic variations within a population are linked to a disease, which can be used to identify genetically-susceptible individuals for that disease.

The finding that CATERPILLER gene products are involved in inflammatory responses, cell survival, and pathogen response point to these polypeptides as new drug targets for identifying compounds for treating inflammatory disease, reducing transplant rejection, enhancing immune responses to vaccines, for reducing abnormal cell growth (e.g., for treating cancer or hyperplasia), for regulating responses to pathogens, and other conditions. Accordingly, in one aspect, the present invention provides methods of identifying a compound or compounds that bind to and/or modulate the activity of a CATERPILLER polypeptide. Any desired end-point can be detected, e.g., binding to the CATERPILLER polypeptide, gene or RNA, modulation of the activity of the CATERPILLER polypeptide, modulation of the Toll-like receptor pathway (e.g., in response to pathogens), modulation of NF- κ B activity, modulation of MHC-II pathway activity and/or interference with binding by a known regulator of a CATERPILLER gene or polypeptide (e.g., TRAF6 and Monarch-1). Methods of detecting the foregoing activities are known in the art and include the methods disclosed herein.

Any compound of interest can be screened according to the present invention. Suitable test compounds include organic and inorganic molecules. Suitable organic molecules can include but are not limited to polypeptides (including enzymes, antibodies and Fab' fragments), carbohydrates, lipids, coenzymes, and nucleic acid molecules (including DNA, RNA and chimerics and analogs thereof) and nucleotides and nucleotide analogs. In particular embodiments, the compound is an antisense nucleic acid, an siRNA or a ribozyme that inhibits production of CATERPILLER polypeptide.

Further, the methods of the invention can be practiced to screen a compound library, e.g., a combinatorial chemical compound library, a polypeptide library, a cDNA library, a library of antisense nucleic acids, and the like, or an arrayed collection of compounds such as polypeptide and nucleic acid arrays.

In one representative embodiment, the invention provides methods of screening test compounds to identify a test compound that binds to a CATERPILLER polypeptide or functional fragment thereof. Compounds that are identified as binding to the CATERPILLER polypeptide or functional

fragment can be subject to further screening (e.g., for modulation of Toll-like receptor pathway activity, for pro- or anti-inflammatory activity, for pro- or anti-apoptosis activity, for modulation of NF- κ B and/or for modulation of MHC-II pathways, and the like) using the methods described herein or other suitable techniques.

Also provided are methods of screening compounds to identify those that modulate the activity of a CATERPILLER polypeptide or functional fragment thereof. The term "modulate" is intended to refer to compounds that enhance (e.g., increase) or inhibit (e.g., reduce) the activity of the CATERPILLER polypeptide (or functional fragment). For example, the interaction of the CATERPILLER polypeptide or functional fragment with a binding party can be evaluated. To illustrate, Monarch-1 is known to bind to TNF α , TRAF6, vimentin, and tubulin among other proteins. As another measure of biological activity, nucleotide binding can be measured. As another alternative, physical methods, such as NMR, can be used to assess biological function. Activity of the CATERPILLER polypeptide or functional fragment can be evaluated by any method known in the art, including the methods disclosed herein.

Compounds that are identified as modulators of CATERPILLER activity can optionally be further screened using the methods described herein (e.g., for binding to the CATERPILLER polypeptide or functional fragment thereof, gene or RNA, modulation of Toll-like receptor pathway activity, for pro- or anti-inflammatory activity, for pro- or anti-apoptosis activity, for modulation of NF- κ B and/or for modulation of MHC-II pathways, and the like). The compound can directly interact with the CATERPILLER polypeptide or functional fragment, gene or mRNA and thereby modulate its activity. Alternatively, the compound can interact with any other polypeptide, nucleic acid or other molecule as long as the interaction results in a modulation of the activity of the CATERPILLER polypeptide or functional fragment.

As another aspect, the invention provides a method of identifying compounds that modulate inflammatory response (i.e., pro- or anti-inflammatory responses). In one representative embodiment, the method comprises contacting a CATERPILLER polypeptide or functional fragment

thereof with a test compound; and detecting whether the test compound binds to the CATERPILLER polypeptide or functional fragment and/or modulates the activity of the CATERPILLER polypeptide (or fragment). In another exemplary embodiment, the method comprises introducing a test compound
5 into a cell that comprises the CATERPILLER polypeptide or functional fragment; and detecting whether the compound binds to the CATERPILLER polypeptide or functional fragment and/or modulates the activity of the CATERPILLER polypeptide or functional fragment in the cell. The CATERPILLER polypeptide can be endogenously produced in the cell.

10 Alternatively or additionally, the cell can be modified to comprise an isolated nucleic acid encoding, and optionally overexpressing, the CATERPILLER polypeptide or functional fragment thereof.

In other representative embodiments, the invention provides a method of identifying a compound that modulates cell survival (both pro- and anti-
15 survival). In one representative embodiment, the method comprises contacting a CATERPILLER polypeptide or functional fragment thereof with a test compound; and detecting whether the test compound binds to the CATERPILLER polypeptide or functional fragment and/or modulates the activity of the CATERPILLER polypeptide/functional fragment. In another
20 exemplary embodiment, the method comprises introducing a test compound into a cell that comprises the CATERPILLER polypeptide or functional fragment thereof; and detecting whether the compound binds to the CATERPILLER polypeptide or functional fragment and/or modulates activity of the CATERPILLER polypeptide/functional fragment in the cell. The
25 CATERPILLER polypeptide can be endogenously produced in the cell. Alternatively or additionally, the cell can be modified to comprise an isolated nucleic acid encoding, and optionally overexpressing, the CATERPILLER polypeptide or functional fragment.

The screening assay can be a cell-based or cell-free assay. Further,
30 the CATERPILLER polypeptide (or functional fragment thereof) or nucleic acid can be free in solution, affixed to a solid support, expressed on a cell surface, or located within a cell.

With respect to cell-free binding assays, test compounds can be synthesized or otherwise affixed to a solid substrate, such as plastic pins, glass slides, plastic wells, and the like. For example, the test compounds can be immobilized utilizing conjugation of biotin and streptavidin by techniques well known in the art. The test compounds are contacted with the CATERPILLER polypeptide or functional fragment thereof and washed. Bound polypeptide can be detected using standard techniques in the art (e.g., by radioactive or fluorescence labeling of the CATERPILLER polypeptide or functional fragment, by ELISA methods, and the like).

Alternatively, the CATERPILLER target can be immobilized to a solid substrate and the test compounds contacted with the bound CATERPILLER polypeptide or functional fragment thereof. Identifying those test compounds that bind to and/or modulate the CATERPILLER polypeptide or functional fragment can be carried out with routine techniques. For example, the test compounds can be immobilized utilizing conjugation of biotin and streptavidin by techniques well known in the art. As another illustrative example, antibodies reactive with the CATERPILLER polypeptide or functional fragment can be bound to the wells of the plate, and the CATERPILLER polypeptide trapped in the wells by antibody conjugation. Preparations of test compounds can be incubated in the CATERPILLER polypeptide (or functional fragment)-presenting wells and the amount of complex trapped in the well can be quantitated.

In another representative embodiment, a fusion protein can be provided which comprises a domain that facilitates binding of the protein to a matrix. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtitre plates, which are then combined with cell lysates (e.g., ³⁵S-labeled) and the test compound, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads are washed to remove any unbound label, and the matrix immobilized and radiolabel detected directly, or in the supernatant after the complexes are dissociated. Alternatively, the complexes can be dissociated from the matrix, separated by

SDS-PAGE, and the level of CATERPILLER polypeptide or functional fragment thereof found in the bead fraction quantitated from the gel using standard electrophoretic techniques.

Another technique for compound screening provides for high
5 throughput screening of compounds having suitable binding affinity to the polypeptide of interest, as described in published PCT application WO84/03564. In this method, a large number of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with the CATERPILLER
10 polypeptide or functional fragment thereof and washed. Bound polypeptide is then detected by methods well known in the art. Purified CATERPILLER polypeptide or a functional fragment can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it
15 on a solid support.

With respect to cell-based assays, any suitable cell can be used including bacteria, yeast, insect cells (e.g., with a baculovirus expression system), avian cells, mammalian cells, or plant cells. In exemplary
20 embodiments, the assay is carried out in a cell line that naturally expresses the CATERPILLER gene or produces the polypeptide. For example, CIAS1 is primarily expressed in monocytic cells, and a monocytic cell line or primary monocytes are suitable for use with CIAS1. Further, in other embodiments, it is desirable to use nontransformed cells (e.g., primary cells) as transformation may alter the function of the polypeptide.

25 The screening assay can be used to detect compounds that bind to or modulate the activity of the native CATERPILLER polypeptide (e.g., polypeptide that is normally produced by the cell). Alternatively, the cell can be modified to express (e.g., overexpress) a recombinant CATERPILLER polypeptide or functional fragment thereof. According to this embodiment, the
30 cell can be transiently or stably transformed with the nucleic acid encoding the CATERPILLER polypeptide or functional fragment, but is preferably stably transformed, for example, by stable integration into the genome of the

organism or by expression from a stably maintained episome (e.g., Epstein Barr Virus derived episomes).

In a cell-based assay, the compound to be screened can interact directly with the CATERPILLER polypeptide or functional fragment thereof (i.e., bind to it) and modulate the activity thereof. Alternatively, the compound can be one that modulates CATERPILLER polypeptide activity (or the activity of a functional fragment) at the nucleic acid level. To illustrate, the compound can modulate transcription of the CATERPILLER gene (or transgene), modulate the accumulation of CATERPILLER mRNA (e.g., by affecting the rate of transcription and/or turnover of the mRNA), and/or modulate the rate and/or amount of translation of the CATERPILLER mRNA transcript.

As a further type of cell-based binding assay, the CATERPILLER polypeptide or functional fragment thereof can be used as a "bait protein" in a two-hybrid or three-hybrid assay (see, e.g., U.S. Pat. No. 5,283,317; Zervos et al., (1993) *Cell* **72**:223-232; Madura et al., (1993) *J. Biol. Chem.* **268**:12046-12054; Bartel et al., (1993) *Biotechniques* **14**:920-924; Iwabuchi et al., (1993) *Oncogene* **8**:1693-1696; and PCT publication WO94/10300), to identify other polypeptides that bind to or interact with the CATERPILLER polypeptide or functional fragment thereof.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the nucleic acid that encodes the CATERPILLER polypeptide or functional fragment thereof is fused to a nucleic acid encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, optionally from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a nucleic acid that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo*, forming a complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter sequence (e.g., LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter can be

detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the nucleic acid encoding the polypeptide that exhibited binding to the CATERPILLER polypeptide or functional fragment.

As another cell-based assay, the invention provides a method of screening a compound for modulation of inflammatory response (including pro- and anti-inflammatory responses). As still another cell-based assay, the invention provides a method of screening a compound for modulation of cell apoptosis (including both pro- and anti-apoptosis). In particular embodiments, the cell comprises an isolated nucleic acid encoding the CATERPILLER polypeptide or functional fragment thereof. According to this embodiment, it is preferred that the isolated nucleic acid encoding the CATERPILLER polypeptide or functional fragment is stably incorporated into the cell (*i.e.*, by stable integration into the genome of the organism or by expression from a stably maintained episome such as Epstein Barr Virus derived episomes). In other methods of the invention, compounds are identified that modulate Toll-like receptor activity, NF- κ B activity and/or MHC-II pathway activity in the cell.

Methods of measuring these activities in cells are known in the art. For example, to measure inflammatory response, NF- κ B, AP-1, JNK and/or p38 activation and/or expression can be measured. The production of products of the inducible nitric oxide synthase (iNOS), *e.g.*, nitric oxide, can also be measured. Cytokine production can also be determined including, but not limited to, production of TNF α , LT α / β , IL-1, IL-4, IL-5, IL-2, IL-6, IL-10, IL-12, IL-18 and IL-23. In addition, phagocytosis of beads, bacteria, other pathogens, and apoptotic or necrotic cells can be used to measure phagocytotic functions. Recognition or killing of immune targets such as antigen presentation function or cell-mediated lympholysis can be used to measure either T cell or antigen presenting cell function. Production of cell-specific products, such as immunoglobulin by B cells, is also a measure of immune activation.

Screening assays can also be carried out *in vivo* in animals. Thus, as still a further aspect, the invention provides a transgenic non-human animal comprising an isolated nucleic acid encoding a CATERPILLER polypeptide or functional fragment thereof, which can be produced according to methods

well-known in the art. The transgenic non-human animal can be from any species, including avians and non-human mammals. According to this aspect of the invention, suitable non-human mammals include mice, rats, rabbits, guinea pigs, goats, sheep, pigs and cattle. Suitable avians include chickens, ducks, geese, quail, turkeys and pheasants.

The nucleic acid encoding the CATERPILLER polypeptide or functional fragment can be stably incorporated into cells within the transgenic animal (typically, by stable integration into the genome or by stably maintained episomal constructs). It is not necessary that every cell contain the transgene, and the animal can be a chimera of modified and unmodified cells, as long as a sufficient number of cells comprise and express the nucleic acid encoding the CATERPILLER polypeptide or functional fragment so that the animal is a useful screening tool.

Exemplary methods of using the transgenic non-human animals of the invention for *in vivo* screening of compounds that modulate inflammatory response (both pro- and anti-inflammatory responses), cell survival (both pro- and anti-survival) and/or the activity of a CATERPILLER polypeptide comprise administering a test compound to a transgenic non-human animal (e.g., a mammal such as a mouse) comprising an isolated nucleic acid encoding a CATERPILLER polypeptide or functional fragment thereof stably incorporated into the genome, administering a test compound to the transgenic non-human animal, and detecting whether the test compound modulates inflammatory response, cell survival and/or CATERPILLER polypeptide activity (or the activity of a functional fragment). Other illustrative methods of the invention can be carried out to identify compounds that modulate MHC-II pathway activity, Toll-like receptor pathway activity, or NF- κ B activity *in vivo*.

It is known in the art how to measure these responses *in vivo*. Illustrative approaches include observation of changes that can be studied by gross examination (edema, redness, swelling, fever, tenderness), histopathology (cellular infiltrates, cell activation markers, phagocytosis, dead cells), changes in cytokine profiles, and cell surface markers (e.g., changes in TNF α , myeloperoxidase or CD69).

Methods of making transgenic animals are known in the art. DNA constructs can be introduced into the germ line of an avian or mammal to make a transgenic animal. For example, one or several copies of the construct can be incorporated into the genome of an embryo by standard
5 transgenic techniques.

In an exemplary embodiment, a transgenic non-human animal is produced by introducing a transgene into the germ line of the non-human animal. Transgenes can be introduced into embryonal target cells at various developmental stages. Different methods are used depending on the stage of
10 development of the embryonal target cell. The specific line(s) of any animal used should, if possible, be selected for general good health, good embryo yields, good pronuclear visibility in the embryo, and good reproductive fitness.

Introduction of the transgene into the embryo can be accomplished by any of a variety of means known in the art such as microinjection,
15 electroporation, lipofection or a viral vector. For example, the transgene can be introduced into a mammal by microinjection of the construct into the pronuclei of the fertilized mammalian egg(s) to cause one or more copies of the construct to be retained in the cells of the developing mammal(s). Following introduction of the transgene construct into the fertilized egg, the
20 egg can be incubated *in vitro* for varying amounts of time, or reimplanted into the surrogate host, or both. One common method is to incubate the embryos *in vitro* for about 1-7 days, depending on the species, and then reimplant them into the surrogate host.

The progeny of the transgenically manipulated embryos can be tested
25 for the presence of the construct by Southern blot analysis of a segment of tissue. An embryo having one or more copies of the exogenous cloned construct stably integrated into the genome can be used to establish a permanent transgenic animal line.

Transgenically altered animals can be assayed after birth for the
30 incorporation of the construct into the genome of the offspring. This can be done by hybridizing a probe corresponding to the DNA sequence coding for the polypeptide or a segment thereof onto chromosomal material from the

progeny. Those progeny found to contain at least one copy of the construct in their genome are grown to maturity.

Methods of producing transgenic avians are also known in the art, see, e.g., U.S. Patent No. 5,162,215.

5 In particular embodiments, to create an animal model in which the activity or expression of a CATERPILLER polypeptide is decreased, it is desirable to inactivate, replace or knock-out the endogenous CATERPILLER gene by homologous recombination with a transgene using embryonic stem
10 acid that upon insertion within or adjacent to the CATERPILLER gene results in a decrease or inactivation of CATERPILLER gene expression or CATERPILLER polypeptide amount or activity.

A knock-out of a CATERPILLER gene means an alteration in the sequence of a CATERPILLER that results in a decrease of function of the
15 CATERPILLER gene, preferably such that the CATERPILLER gene expression or CATERPILLER polypeptide amount or activity is undetectable or insignificant. Knock-outs as used herein also include conditional knock-outs, where alteration of the CATERPILLER gene can occur upon, for example, exposure of the animal to a substance that promotes
20 CATERPILLER gene alteration, introduction of an enzyme that promotes recombination at a CATERPILLER gene site (e.g., Cre in the Cre-lox system), or other method for directing the CATERPILLER gene alteration postnatally. Knock-out animals may be prepared using methods known to those of skill in the art. See, for example, Hogan, *et al.* (1986) *Manipulating the Mouse*
25 *Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

A knock-out construct is a nucleic acid sequence, such as a DNA construct, which, when introduced into a cell, results in suppression (partial or complete) of expression of a polypeptide encoded by endogenous DNA in the
30 cell. A knock-out construct as used herein may include a construct containing a first fragment from the 5' end of the CATERPILLER gene, a second fragment from the 3' end of the CATERPILLER gene and a DNA fragment encoding a selectable marker positioned between the first and second

CATERPILLER fragments. It should be understood by the skilled artisan that any suitable 5' and 3' fragments of a CATERPILLER gene may be used as long as the expression of the corresponding CATERPILLER gene is partially or completely suppressed by insertion of the transgene. Suitable selectable markers include, but are not limited to, neomycin, puromycin and hygromycin. In addition, the construct may contain a marker, such as diphtheria toxin A or thymidine kinase, for increasing the frequency of obtaining correctly targeted cells. Suitable vectors include, but are not limited to, pBLUESCRIPT, pBR322, and pGEM7.

Alternatively, a knock-out construct may contain RNA molecules such as antisense RNA, siRNA and the like to decrease the expression of a CATERPILLER gene. In particular embodiments, the siRNA molecules comprise **SEQ ID NO:122** and/or **SEQ ID NO:123** (Monarch-1), **SEQ ID NO:133** (CATERPILLER 11.2) or **SEQ ID NO:143** and/or **SEQ ID NO:144** (CATERPILLER 16.2). Typically, for stable expression the RNA molecule is placed under the control of a promoter. The promoter may be regulated, if deficiencies in the protein of interest may lead to a lethal phenotype, or the promoter may drive constitutive expression of the RNA molecule such that the gene of interest is silenced under all conditions of growth. While homologous recombination between the knock-out construct and the CATERPILLER gene of interest may not be necessary when using an RNA molecule to decrease CATERPILLER gene expression, it may be advantageous to target the knock-out construct to a particular location in the genome of the host organism so that unintended phenotypes are not generated by random insertion of the knock-out construct.

The knock-out construct may subsequently be incorporated into a viral or nonviral vector for delivery to the host animal or may be introduced into embryonic stem (ES) cells. ES are typically selected for their ability to integrate into and become part of the germ line of a developing embryo so as to create germ line transmission of the knock-out construct. Thus, any ES cell line that can do so is suitable for use herein. Suitable cell lines which may be used include, but are not limited to, the 129J ES cell line or the J1 ES cell line. The cells are cultured and prepared for DNA insertion using methods well-

- known to the skilled artisan (e.g., see Robertson (1987) *In: Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. IRL Press, Washington, D.C.; Bradley, et al. (1986) *Curr. Topics Develop. Biol.* 20:357-371; Hogan, et al. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

- Insertion of the knock-out construct into the ES cells may be accomplished using a variety of methods well-known in the art, including, for example, electroporation, microinjection, and calcium phosphate treatment.
- For insertion of the DNA or RNA sequence, the knock-out construct nucleic acids are added to the ES cells under appropriate conditions for the insertion method chosen. If the cells are to be electroporated, the ES cells and construct nucleic acids are exposed to an electric pulse using an electroporation machine (electroporator) and following the manufacturer's guidelines for use. After electroporation, the cells are allowed to recover under suitable incubation conditions. The cells are then screened for the presence of the knockout construct.

- Each knock-out construct to be introduced into the cell is first typically linearized if the knock-out construct has been inserted into a vector.
- Linearization is accomplished by digesting the knock-out construct with a suitable restriction endonuclease selected to cut only within the vector sequence and not within the knock-out construct sequence.

- Screening for cells which contain the knock-out construct (homologous recombinants) may be done using a variety of methods. For example, as described herein, cells can be processed as needed to render DNA in them available for hybridization with a nucleic acid probe designed to hybridize only to cells containing the construct. For example, cellular DNA can be probed with ³²P-labelled DNA which locates outside the targeting fragment. This technique can be used to identify those cells with proper integration of the knock-out construct. The DNA can be extracted from the cells using standard methods (e.g., see, Sambrook, et al. (1989) *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The DNA may then be analyzed by Southern blot with a probe

or probes designed to hybridize in a specific pattern to genomic DNA digested with one or more particular restriction enzymes.

Once appropriate ES cells are identified, they are introduced into an embryo using standard methods. They can be introduced using
5 microinjection, for example. Embryos at the proper stage of development for integration of the ES cell to occur are obtained, such as by perfusion of the uterus of pregnant females. For example, mouse embryos at 3-4 days development can be obtained and injected with ES cells using a micropipet. After introduction of the ES cell into the embryo, the embryo is introduced into
10 the uterus of a pseudopregnant female mouse. The stage of the pseudopregnancy is selected to enhance the chance of successful implantation. In mice, 2-3 days pseudopregnant females are appropriate.

Germline transmission of the knockout construct may be determined using standard methods. Offspring resulting from implantation of embryos
15 containing the ES cells described above are screened for the presence of the desired alteration (e.g., CATERPILLER knock-out). This may be done, for example, by obtaining DNA from offspring (e.g., tail DNA) to assess for the knock-out construct, using known methods (e.g., Southern analysis, dot blot analysis, PCR analysis). See, for example, Sambrook, et al. (1989) Molecular
20 Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). Offspring identified as chimeras may be crossed with one another to produce homozygous knock-out animals.

Mice are often used as animal models because they are easy to house, relatively inexpensive, and easy to breed. However, other knock-out animals
25 may also be made in accordance with the present invention such as, but not limited to, monkeys, cattle, sheep, pigs, goats, horses, dogs, cats, guinea pigs, rabbits and rats. Accordingly, appropriate vectors and promoters well-known in the art may be selected and used to generate a transgenic animal deficient in CATERPILLAR expression.

30 Particular embodiments of the present invention are described in greater detail in the following non-limiting examples.

EXAMPLE 1

**Identification of Mammalian Genes Containing CARD,
Pyrin, Nucleotide Binding, and LRR Domains**

This example describes the identification of twenty-two known and
5 novel NBD/LRR genes which are spread across 8 human chromosomes, with
multi-gene clusters occurring on chromosomes 11, 16, and 19. The N-termini
of these proteins vary, but most have a pyrin domain. The genomic
organization demonstrates a high degree of conservation in the nucleotide-
binding domain (NBD) and C-terminal leucine-rich repeat (LRR) encoding
10 exons. Except for CIITA, all the predicted NBD/LRR proteins appear to
contain an ATP-binding domain. Some have broad tissue expression, while
others are restricted to immune cells.

Materials and Methods

15 *Databases and Search Strategies.* Searches were performed using the
published Celera human genome scaffold data (Venter, *et al.* (2001) *Science*
291:1304); the NCBI "nr" database, containing GENBANK®, EMBL, DDBJ,
PDB, and completed phase 3 and 4 high-throughput genomic sequencing
(HTGS) sequences; and the NCBI genome database (Lander, *et al.* (2001)
20 *Nature* 409:860). Initial searches were performed using the B cell form of
CIITA protein sequence (Steimle, *et al.* (1993) *Cell* 75:135) as a query
employing the BLAST search algorithms BLASTP and TBLASTN (**Figure 1**).
BLASTP identifies amino acid sequence similarities through query sequence
comparison with database proteins and is more likely to find distant
25 relationships than BLASTN (Pearson (2000) *Methods Mol. Biol.* 132:185).
TBLASTN compares the query protein sequence with translations of all six
reading frames of available nucleotide sequences and has the same
advantages as BLASTP. Analogous domains of the resultant sequences were
employed to identify additional sequences and/or confirm initial identities, this
30 is known as DOUBLE-BLAST inspired by the ISS method (Park, *et al.* (1998)
J. Mol. Biol. 284:1201; Karplus, *et al.* (1998) *Bioinformatics* 14:846) and is
comparable in homolog detection to Hidden Markov Methods. LRR
sequences, the N-terminal pyrin domains of DEFCAP, and the caspase

recruitment domains (CARD) of Nod1 and Nod2 were used to perform similar searches. The N-terminal sequences of CIITA yielded no related sequences obviously belonging to an NBD/LRR protein.

5 *Assembly of Putative Novel Genes and Construction of Genomic Maps.* Pyrin and LRR sequences identified within contigs containing NBDs were examined for location and orientation to determine the likelihood of residing in the same operon as an identified NBD. Pyrin and LRR domains were considered contiguous with an NBD if they fell upstream and
10 downstream of the NBD, respectively, in the same orientation. CARD domains occur both upstream (Nod1/2) and downstream (DEFCAP) of the NBD (Hlaing, *et al.* (2001) *J Biol Chem* 276:9230), but none of the novel sequences contained CARD domains. As sequence data became available for more than a single domain, a putative genomic organization was
15 generated by comparing the "cDNA" sequence to the genome sequence.

Cell Lines, Preparation of RNA, and RT-PCR. HeLa, MCF7, Jurkat, Raji, and RAMOS cell lines were cultured in either Dulbecco's Modified Eagle Medium (DMEM) (high glucose) or RPMI1640 with 10% fetal calf serum, L-
20 glutamine, and penicillin/streptomycin. Peripheral blood leukocytes were obtained as buffy coats from the American Red Cross (Durham, NC). Total RNA was prepared using the SV Total RNA Isolation Kit (PROMEGA™, Madison, WI). Total RNA was reversed transcribed to cDNA using MMLV reverse transcriptase and amplified in an MJ Thermocycler (MJ Research,
25 San Francisco, CA) in a separate reaction with primers specific for each target sequence. Amplification products were electrophoresed on 0.8% agarose and visualized with ethidium bromide.

Experimental Results

30 *Identification of Novel CIITA-Related Sequences.* BLAST searches of the published Celera and NCBI genomic databases using the NBD and LRR of CIITA, Nod1, Nod2, DEFCAP and resultant target sequences as queries revealed 22 potential genes and pseudogenes, including the presently known

genes, unified by the presence of an NBD and downstream LRRs (Table 1). New genes were assigned a name based on chromosome number and order of discovery (e.g. 19.1, first found on chr. 19). Nod1, Nod2, and DEFCAP contain CARD domains which may be involved in recruiting caspases (Inohara, *et al.* (1999) *J. Biol. Chem.* 274:14560; Ogura, *et al.* (2001) *J. Biol. Chem.* 276:4812; Hlaing, *et al.* (2001) *supra*). DEFCAP also has an N-terminal Pyrin domain with homology to the familial Mediterranean fever protein (Bertin and DiStefano (2000) *Cell Death Differ.* 7:1273). BLAST searches were also performed for the CARD domains of Nod1/2, the pyrin domain of DEFCAP, and resulting target sequences. CARD domain homologs were not found for any of the novel sequences. The majority of the putative genes had upstream Pyrin domains, but the upstream N-terminal sequences of several remain unknown.

TABLE 1

	N-terminus	P-loop (Kinase 1/G1) ¹
1.1/CIAS1	Pyrin	GAAGIGKT (SEQ ID NO:37)
Nod1	CARD	GDAGVGKS (SEQ ID NO:38)
11.1	Pyrin	GSAGTGKT (SEQ ID NO:39)
11.2	Pyrin	GAAGVGKT (SEQ ID NO:40)
11.4	Pyrin	GPAGTGKT (SEQ ID NO:41)
11.3	?	GTVGTGKS (SEQ ID NO:42)
12	Pyrin	None
CIITA	CARD, Acidic	GKAGQGKS (SEQ ID NO:43)
Nod2	CARD x2	GEAGSGKS (SEQ ID NO:44)
16.1	?	GKAGMGKT (SEQ ID NO:45)
16.2	?	GVAGMGKT (SEQ ID NO:46)
Nalp1/DEFCA P	Pyrin	GAAGIGKS (SEQ ID NO:47)
Nalp2	Pyrin	GPAGLGKT (SEQ ID NO:48)
19.1	?	GPDGIGKT (SEQ ID NO:49)
19.2	Pyrin	GAPGIGKT (SEQ ID NO:50)
19.3	Pyrin	GAAGIGKS (SEQ ID NO:51)
19.4	Pyrin	GPAGVGKT (SEQ ID NO:52)

19.5	Pyrin x2	GPQGIGKT (SEQ ID NO:53)
19.6	Pyrin	GERASGKT (SEQ ID NO:54)
19.7	Pyrin	GRAGVGKT (SEQ ID NO:55)
19.8	?	GKSGIGKS (SEQ ID NO:56)
X	?	ACAGTGKT (SEQ ID NO:57)
Apaf1		GMAGCGKS (SEQ ID NO:58)
RPM1		GMGGSGKT (SEQ ID NO:59)
NAIP		GEAGSGKT (SEQ ID NO:60)
HET-E		GDPGKGKT (SEQ ID NO:61)
TP1		GQSGQGKT (SEQ ID NO:62)
G alpha 12		GAGESGKS (SEQ ID NO:63)

	GTP-Mg ⁺² (G3) ²	ATP-Mg ⁺² (Kinase 2) ³
1.1/CIAS1	-	LFLMD (SEQ ID NO:74)
Nod1	-	LFTFD (SEQ ID NO:75)
11.1	-	LFILD (SEQ ID NO:76)
11.2	-	LFIID (SEQ ID NO:77)
11.4	-	LFILD (SEQ ID NO:76)
11.3	-	-
12	-	LFIMD (SEQ ID NO:78)
CIITA	DAYG (SEQ ID NO:64)	LLILD (SEQ ID NO:79)
Nod2	-	LLTFD (SEQ ID NO:80)
16.1	-	LLIFD (SEQ ID NO:81)
16.2	-	LLILD (SEQ ID NO:79)
Nalp1/DEFCAP	DEPG ⁶ (SEQ ID NO:65)	LFILD (SEQ ID NO:76)
Nalp2	DELG ⁶ (SEQ ID NO:66)	LFVID (SEQ ID NO:82)
19.1	-	LFIMD (SEQ ID NO:78)
19.2	-	LLLLD (SEQ ID NO:83)
19.3	-	LFIID (SEQ ID NO:77)
19.4	DICG ⁶ (SEQ ID NO:67)	LFVID (SEQ ID NO:82)
19.5	-	LFVID (SEQ ID NO:82)
19.6	-	LFILD (SEQ ID NO:84)
19.7	-	LFIID (SEQ ID NO:77)
19.8	DDLG ⁶ (SEQ ID NO:68)	LFIID (SEQ ID NO:77)
X	DPVG ⁶ (SEQ ID NO:69)	LLILD (SEQ ID NO:79)
Apaf1	DKSG (SEQ ID NO:70)	LLILD (SEQ ID NO:79)
RPM1	-	IVVLD (SEQ ID NO:85)

NAIP	-	LFLLD (SEQ ID NO:86)
HET-E	DHAG (SEQ ID NO:71)	YLIID (SEQ ID NO:87)
TP1	DQNG ⁶ (SEQ ID NO:72)	VLIID (SEQ ID NO:88)
G alpha 12	DKLG (SEQ ID NO:73)	-

	Guanine Binding (G4) ⁴	Nucleotide Specificity ⁵	LRR
1.1/CIAS1	-	ATP	Duplex
Nod1	-	ATP	Single
11.1	-	ATP	Single
11.2	-	ATP	Duplex
11.4	-	ATP	Duplex
11.3	-	-	Non-Uniform
12	-	-	Single/Duplex
CIITA	SKAD (SEQ ID NO:89)	GTP ⁷	Single
Nod2	-	ATP	Single
16.1	-	ATP	Single
16.2	-	ATP	Single
Nalp1/DEFBAP	-	ATP	Single/Duplex
Nalp2	-	ATP	Duplex
19.1	-	ATP	Duplex
19.2	-	ATP	Duplex
19.3	-	ATP	Duplex
19.4	-	ATP	Duplex
19.5	-	ATP	Duplex
19.6	-	ATP	Duplex
19.7	-	ATP	Duplex
19.8	-	ATP	Duplex
X	-	-	Duplex
Apaf1	-	dATP ⁷ /ATP ⁷	WD40
RPM1	-	ATP	LRR
NAIP	-	ATP	LRR
HET-E	TKHD (SEQ ID NO:90)	GTP/ATP	WD40
TP1	-	ATP	WD40
G alpha 12	SKQD (SEQ ID NO:91)	GTP ⁷	-

¹Consensus P-loop motif, GXXXXGK(S/T (SEQ ID NO:92)); ²Consensus Mg+2 site (G3), DXXG (SEQ ID NO:93); ³Consensus Mg+2 site (Kinase2), ψψψψD (SEQ ID NO:94), ψ=hydrophobic; ⁴Consensus Guanine-binding site (G4), (N/T/S)KXD (SEQ ID NO:95); ⁵Predicted nucleotide specificity; ⁶G3 motif occurring after kinase2; ⁷Published nucleotide specificity. NAIP, CIITA, HET-E, and TP1 are the defining members of the NACHT family. Apaf1, RPM1, NAIP, HET-E, TP1, and G alpha 12 are shown for comparison purposes. Pseudogenes and suspected pseudogenes are shown in italics.

Conservation of Intron-Exon Organization. Exon/intron sizes and positions were determined for the known and some predicted NBD/LRR proteins by the location of the sequence corresponding to the mRNA/cDNA, assuming intactness of the contig (**Figure 2A**). The genomic organization was complex and remarkably similar for all of the sequences examined, with large NBD exons (~1500 nucleotides) and LRR exons of about 76 nucleotides, 174 nucleotides, or both, depending on the gene. CARD and Pyrin domains were approximately 300 nucleotides long.

CATERPILLER Domains. Distinct domains of each sequence are provided in **Table 1**. Nod1, Nod2, and CIITA had N-terminal CARD or CARD-like domains. Thirteen had N-terminal Pyrin domains. CIITA was unique in having an N-terminal acidic transactivation domain. Five of these sequences did not have CARD, Pyrin, or CIITA-like activation domains upstream of their NBDs. The diversity of these N-terminal sequences indicates multiple functional modes.

The predicted nucleotide specificity based on motifs found in the CATERPILLER genes is also provided in **Table 1**. This was compared to another family, containing plant and animal proteins, grouped on the basis of an NTPase domain and C-terminal repeats of either the LRR or WD40 type, called the NACHT family which includes NAIP, CIITA, HET-E and TP1 (Koonin and Aravind (2000) *Trends Biochem. Sci.* 25:223). The majority were predicted to be ATP binding proteins, with the exception of CIITA, which binds GTP, and HET-E. A GTP-binding protein-like magnesium coordination (G3) motif (DXXG; SEQ ID NO:93) occurred in a number of the other sequences, with the exception of the more distantly related Apaf1, it followed the more typical Kinase 2 site found in ATP-binding proteins.

The NBDs of these predicted proteins were aligned, each approximately 500 amino acids in length, and twelve groupings of conserved residues (motifs) were observed (**Figure 2B, Figures 3A-G**). While the seven NACHT motifs were present, the larger number of compared sequences permitted a refined definition of the NACHT domain that excluded WD40

repeat-containing members thus distinguishing a CATERPILLER NBD from the broader NACHT family. These motif definitions also indicated a divergence between the majority of the NBDs provided herein and those like NAIP. Functionally important motifs may include motif I, which contains the Walker A sequence found in most nucleotide binding proteins (Traut (1994) *Eur. J. Biochem.* 222:9), and Motif III and V that overlap with or are adjacent to leucine-charged domain (LCD) motifs (Heery, *et al.* (1997) *Nature* 387:733); motifs important for ClITA function (Harton and Ting (2000) *Mol. Cell Biol.* 20:6185). Motif IV contains the Kinase 2 motif which coordinates magnesium ions in ATP binding proteins (Traut (1994) *supra*).

The presence of LRR sequences downstream of the NBD was required for inclusion as a CATERPILLER family member. The LRR sequences following NBDs had two exon arrangements, a "singlet" (~74 nucleotides) containing one motif iteration or a "duplex" (~180 nucleotides) containing two (Table 1, last column; Figure 2A; Figures 3A-G). The sole requirement for inclusion as an LRR was conservation of the hydrophobic residues "leucines" comprising the motif. It is important to note that BLAST searches for LRRs may miss some sequences due to a greater likelihood of less similarity between non-LRR-motif residues. Thus, without actual cDNA clones it was impossible to be highly confident that all of the LRR exons downstream of the NBD had been identified for each putative gene. Given this caveat, it appears that all of the genes on chromosome 19 had doublet LRR exons while those on chromosome 16 had singlets. DEFCAP and the potential pseudogene 12, had both singlet and doublet exons.

Phylogenetic Analysis of the NBD and Evolutionary Issues. An analysis using protein alignment and tree generation software (Data Analysis in Molecular Biology and Evolution; Xia and Xie (2001) *J. Hered.* 92:371) was performed to examine the potential phylogenetic relationship of the predicted NBD protein sequences (Figure 4). Apaf1 and RPM1 (Table 1) were included as their NBD regions were similar to this family. Except for 11.3, the newly-identified NBD sequences were more closely related to one another than Apaf1 (Figure 4), indicating that NBD/WD40-repeat proteins were more

distantly related. The NBD of RPM1, an NBD/LRR R protein of *Arabidopsis*, was most closely related to Apaf1. The novel NBD most closely related to RPM1 was 11.3 which has an NBD exon interrupted by an intron. Consistent with divergent evolution, the NBDs of the known and putative proteins with upstream CARD domains were more closely related to each other than to those NBDs with upstream Pyrin domains which form their own grouping phylogenetically.

The assignment of the CATERPILLER genes to chromosomal positions is shown in **Figure 5**. Most were found in clusters on chromosomes 11, 16, and 19. Three occur at 11p15, three more between 16p12 and 16p13, and nine at 19q13. Proximities of the six sequences on a single contig at 19q13.4, indicates that gene duplication had occurred for these sequences. With the exception of four of these sequences, all were near the telomere, indicating that those found singly may have their origins in chromosomal recombination. Among those not at the telomeric end of chromosomes, one (X) may be a pseudogene. In *Saccharomyces*, fermentation gene alleles are thought to have been generated by the duplication of genes close to the telomeric end and subsequent genomic dispersion by recombination (Charron, *et al.* (1989) *Genetics* 122:307).

The presence of multiple individual exons containing one or two LRR indicates that exon shuffling may occur and that natural selection may favor the maintenance or elimination of a given LRR sequence or pair while simultaneously preserving other aspects of the gene in question. The specificity of plant R proteins is principally dependent upon the LRR and these are targets for diversifying selection (Dangl and Jones (2001) *Nature* 411:826). In Flax, a six amino acid difference in the LRR of P versus P2 determines Rust R protein specificity (Dodds, *et al.* (2001) *Plant Cell* 13:163). The LRRs of RPS2 contain a small stretch important for cooperation with host factors determining *Arabidopsis* resistance to *Pseudomonas syringae* (Banerjee, *et al.* (2001) *Genetics* 158:439). Unequal recombination, gene conversion, and accumulated mutations may generate novel specificities for the NBD/LRR class of R proteins.

Evidence for Expression of the CATERPILLER Genes. Information available on the expression patterns of the known genes was available and reflected their biologic role. CIITA has three different isoforms arising from three different promoters. Nod1 has a wide tissue distribution (Inohara, et al. (1999) *supra*), while Nod2 and CIAS1 are restricted to monocytes, consistent with inflammatory roles (Hoffman, et al. (2001) *Nat. Genet.* 29:301; Ogura, et al. (2001) *supra*). The expression of the other sequences was examined by using the NCBI database to search for expressed sequence tags encoding at least part of the sequence (**Table 2**). UniGene sequence entries existed for CIAS1, Nod1, Nod2, DEFCAP, Nalp2, and 16.1. Fourteen of the genes were represented in the GENBANK® human est database. The gene identified herein as 19.3, also referred to herein as Monarch-1, has been previously described as a partial cDNA encoding a 344 amino acid protein (RNO2) comprised of leucine-rich repeats and is expressed in bone marrow, peripheral blood leukocytes, and nitric oxide treated HL-60 cells (Shami, et al. (2001) *Br. J. Haematol.* 112:138). No est entry was found for 11.2, 12, 19.1, 19.2, 19.5, 19.8, or X. A survey of the expression of these new genes was conducted and is summarized in **Table 2**. Message was detected for every non-pseudogene except 19.1 and 19.2. Nearly all of the family members were expressed in hematopoietic cells and are likely restricted as ubiquitous expression was uncommon.

TABLE 2

Name	UniGene	Genbank EST	Hematopoietic ¹	Somatic ²
1.1/ CIAS1	Hs.159483	+	+	-
Nod1	Hs.19405	+	+ ³	+ ³
11.1		+	+	+
11.2			+	-
11.3		+	+	+
11.4		+	+	-
12			NT	NT
CIITA		+	+ ³	+ ^{3,4}
Nod2	Hs.135201	+	+ ³	- ³
16.1	Hs.10888	+	+	+
16.2		+	+	-
DEFCAP	Hs.104305	+	+	+
19.1			-	-
19.2			-	-
19.3		+	+	
19.5			+	-
19.6		+	+	-
19.7		+	+	-
19.8			+	-
Nalp2/19.	Hs.6844	+		
4			+	-
X			NT	NT

For EST searches, stretches of significant identity to translated EST sequences were considered a positive match.

¹Primary human hematopoietic cells or cell lines. ²HeLa and MCF7 (non-small cell lung carcinoma). ³From published sources. ⁴When induced. Expression was determined by reverse transcriptase-PCR using cDNA derived from the indicated sources. NT = not tested.

Immunologic Significance. Of the known genes, CIITA, CIAS1, and Nod2 are clearly linked to immune function. CIITA directly controls major histocompatibility complex II (MHC II) gene expression, whereas CIAS1 in familial cold urticaria and Nod2 in Crohn's disease are likely regulating inflammatory responses. DEFCAP and Nod1 both promote apoptosis and activate NF- κ B. Activation of NF- κ B is also observed for Nod2, and under appropriate conditions for CIAS1. These functions are reminiscent of plant R

proteins that promote plant responses similar to innate immune functions (Dangl and Jones (2001) *supra*).

Innate immune responses mediated by Toll in response to fungal pathogens in *Drosophila* highlight the importance of receptors recognizing specific pathogen-associated molecular patterns (Medzhitov (2001) *Nature Rev. Immunol.* 1:135). LRR-containing proteins in plants and animals serve a similar function which is supported by the threading result with selected LRRs indicating that LRR structural features are conserved in the NBD/LRR family. Toll-like Receptors have extracellular LRRs mediating recognition of a variety of microbial derivatives (Poltorak, *et al.* (2000) *Proc. Natl. Acad. Sci. U S A* 97:2163; Bauer, *et al.* (2001) *Proc. Natl. Acad. Sci. USA* 98:9237). The LRRs of plant R proteins likewise recognize avirulence proteins from plant pathogens and provide specificity (Van Der Hoorn, *et al.* (2001) *Plant Cell* 13:273). Recent studies of Nod1 and Nod2 demonstrate that both require their LRRs for responses to various bacterial LPSs (Inohara, *et al.* (2001) *J. Biol. Chem.* 276:2551). CLITA's LRRs, while not known to interact with any pathogen-specific molecule, are functionally necessary, involved in self-association, interaction with an endogenous protein, and regulation of nuclear import (Linhoff *et al.* (2001) *MCB* 21:3001; Harton *et al.*, (2002) *Hum. Immunol.* 63:588). Thus, these LRRs may serve as versatile recognition domains with specificity for self-interaction, protein/lipid/sugar recognition, or both. Deletion of the LRRs from Nod1/2, DEFCAP, and CIAS1 enhances their activities indicating that these LRRs are important sites of regulation.

NBD/LRR Genes in Other Organisms. The number of identified mammalian NBD/LRR sequences was significantly smaller than that occurring in some plants (Pan, *et al.* (2000) *J. Mol. Evol.* 50:203). The mammalian family may be larger than described herein as NAIP and Ipaf (CARD12), despite having NBDs and LRRs, were not detected using the parameters of this study, likely due to the absence of some of the CATERPILLER motifs in their NBDs. Limited BLAST searches of translated nucleotide sequences from *Drosophila* and *C. elegans* genomic databases failed to identify any NBD/LRR genes. A similar search of the *Danio rerio* (zebrafish) database did

yield likely NBD/LRR sequences and the mouse genome had at least as many genes in this family as humans. The preponderance of NBD/LRR proteins in plants may be due to reliance upon individual effector molecules for recognizing pathogen-specific products. Higher order eukaryotes have developed a highly complex adaptive immune system driving a staggering array of protein-specific immune responses with a limited number of genes.

N-terminal variation in the known and predicted genes indicates a subdivision of CATERPILLER proteins; Group I, CARD-containing (e.g., Nod1); Group II, Pyrin-containing (e.g., DEFCAP); Group III, transactivation domain (e.g., CIITA); and unknown (e.g., 16.1) (Table 1). However these grouping may be oversimplified. For example, multiple cell-type-specific forms of CIITA are known. The dendritic cell form has a CARD-like N-terminus followed by the activation domain, although no caspase-recruitment activity has been described (Nickerson, *et al.* (2001) *J. Biol. Chem.* 276:19089). Nod2 and cryopyrin are also expressed as multiple transcripts (Hoffman, *et al.* (2001) *supra*; Ogura, *et al.* (2001) *supra*). In addition, self-association has been demonstrated for CIITA and Nod1, while heterodimerization of CIAS1 with apoptotic protein ASC may involve CIAS1's pyrin domain (Manji, *et al.* (2002) *J. Biol. Chem.* 277:11570; Ting and Trowsdale (2002) *Cell* 109 Suppl:S21; Inohara, *et al.* (1999) *supra*). Self- and hetero-association may amplify and generate diversity necessary to mediate appropriate responses.

Six of the CATERPILLAR genes predicted herein were cloned and more fully characterized in detail in the following examples.

EXAMPLE 2

Characterization and Functional Analysis of CATERPILLER Monarch-1

This example describes the characterization of nucleic acid sequences encoding murine and human Monarch-1 proteins. Monarch-1 has four different splice forms due to the differential splicing of LRR motifs. The nucleic acid sequences encoding a full-length human Monarch-1 protein sequence are set forth as SEQ ID NO:1 and SEQ ID NO:2, respectively. The nucleic

acid and protein sequences of a human splice isoform II are set forth as SEQ ID NO:3 and SEQ ID NO:4. The nucleic acid and protein sequences of a human splice isoform III are set forth as SEQ ID NO:5 and SEQ ID NO:6. The nucleic acid and protein sequences of a human splice isoform IV are set forth as SEQ ID NO:7 and SEQ ID NO:8. It has now been found that Monarch-1 is expressed by immune cells; is part of the endotoxin tolerant pathway; inhibits cellular responses induced by endotoxin from bacteria; inhibits IFI16, an interferon responsive protein; causes changes in cytokine mRNA (TNF- α and IL-10) expression; functions in the enhancement of class I MHC gene expression; enhances IL-6, IL-10 and IL-1 β cytokine production; and interacts with tubulin, vimentin, hsp-70, TNIK, NIK, CARD10, TRAF6 and CIAS1. The results provided herein indicate that Monarch-1 regulates molecules important in the inflammatory response and cell survival.

Materials and Methods

Reagents. [(Z)-1-[2-(2-Aminoethyl)-N-(2-ammonioethyl)amino]diazen-1-yl]-1,2-diolate] (Deta-NO), an inducer of nitric oxide, was used at 125 μ mol/l (Alexis Biochemicals, San Diego, CA). IFN γ was used at 1000 U/ml, TGF β at 1 ng/ml, TNF α at 20 ng/ml (Peprotech, Rocky Hill, NJ) and phorbol 12-myristate 13-acetate (PMA) (Sigma, St. Louis, MO) at 10 ng/ml.

Cell Lines. HeLa cells were transfected with 1 μ g of pcDNA3-HA vector or HA-tagged Monarch-1 via FUGENE® (Roche, Indianapolis, IN) and selected with 500 μ g/ml G418. U937 siRNA clones were selected with 500 μ g/ml puromycin.

Cell Preparation and Purification. BMC were isolated from buffy coats (American Red Cross, Durham, NC) using Lymphocyte Separation Media (ICN, Costa Mesa, CA). T cells, B cells, monocytes and CD15+ granulocytes were individually selected by a magnetic-activated cell sorting (MACS) column (Miltenyi Biotech, Auburn, CA). Monocyte-derived dendritic cells were generated by differentiating peripheral blood mononuclear cells (PBMCs) with

GM-CSF and IL-4 for 8 days.

TLR Luciferase Reporter Gene Assays. HEK293T cells were plated at 1×10^4 cells/well in 96-well plates and transfected the following day using
5 FUGENE™ transfection reagent (Roche, Indianapolis IN) in accordance with the manufacturer's recommendations. Cells were transfected with 50 ng of NF- κ B-luc reporter and 200 ng of vector, MyD88, TRAF6 or IRAK1 expression plasmids to induce NF- κ B activity. Cells were co-transfected with either 400 ng of vector or Monarch-1 expression plasmid. Amounts of the relevant
10 expression plasmids were transfected as indicated, maintaining the total amount of DNA constant using pcDNA3 empty vector. Cells were harvested 36 hours after transfection and equal amounts of protein were assayed for luciferase activity following standard procedures. Equal protein amounts were determined using the Bradford protein assay (BIO-RAD®, Hercules, CA).

15 *RNA Preparation and Real-Time PCR.* Total RNA was isolated using the SV40 Total RNA System (PROMEGA™, Madison, WI) with an additional DNase I digestion step. Real-Time PCR was performed with the TAQMAN® sequence detection system (Applied Biosystems, Foster City, CA). Primers
20 and probes for mouse Monarch-1 were: forward 5'-TGCTACAAGTCCGGGACAAA-3' (SEQ ID NO:96); reverse 5'-GCCCAGTTCTGGGTCATTT-3' (SEQ ID NO:97); and probe 5'-CAGCAGAGCCTCAGAGTGCTTCG-3' (SEQ ID NO:98). Primers and probe for 18S ribosomal RNA were: forward 5'-GCTGCTGGCACCAGACTT-3' (SEQ
25 ID NO:99); reverse 5'-CGGCTACCACATCCAAGG-3' (SEQ ID NO:100); and probe 5'-CAAATTACCCACTCCCGACCCG-3' (SEQ ID NO:101). Primers and probe for HLA-G were: forward 5'-AGACCCTGCCGCGCTACT-3' (SEQ ID NO:102); reverse 5'-TCCACTGGAGGGTGTGAGAAC-3' (SEQ ID NO:103); and probe 5'-AACCAGAGCGAGGCC-3' (SEQ ID NO:104). Primers and
30 probe for HLA-B were: forward 5'-GGGACCGGGAGACACAGAT-3' (SEQ ID NO:105); reverse 5'-GCGCAGGTTCTCTCGGTAAG-3' (SEQ ID NO:106); and probe 5'-CAAGACCAACACACAG-3' (SEQ ID NO:107). Primers and probe for LMP7b were: forward 5'-GCCGCAGGGCTATTGCTTA-3' (SEQ ID NO:108);

reverse 5'-CATATTGACAACGCCTCCAGAA-3' (SEQ ID NO:109); and probe 5'-CACTCACAGAGACAGCT-3' (SEQ ID NO:110). Primers and probe for GAPDH were: forward 5'-ACCTCAACTACATGGTTTAC-3' (SEQ ID NO:111); reverse 5'-GAAGATGGTGGTGGGATTTTC-3' (SEQ ID NO:112); and probe 5'-CAAGCTTCCCGTTCTCAGCC-3' (SEQ ID NO:113). Results were normalized to the GAPDH mRNA and 18S ribosomal RNA internal controls and were expressed in relative numbers.

Monarch-1 RT-PCR. To clone the N-terminal region of human Monarch-1, the following primers were used: Monarch-1 N-term forward 5'-GGGGTACCGCTACGAACCGCAGGCAGGGACG-3' (SEQ ID NO:114); Monarch-1 N-term reverse 5'-CAGCCTGGTCACGTCCTGGTCTG-3' (SEQ ID NO:115). To clone the suspected C-terminal region and identify LRR splice forms, the following primers were used: Monarch-1 C-term forward 5'-CAGAAGGACATCAACTGTGAGAG-3' (SEQ ID NO:116); Monarch-1 C-term reverse 5'-GCTCTAGACAGCAGATAGGACCATTCAGCAG-3' (SEQ ID NO:117). The One-Step RT-PCR Kit (QIAGEN®, Valencia, CA) was employed following the manufacturer's instructions. For expression analysis, the primers used were Monarch-1 pyr-NBD forward 5'-TTGAGCGGATAAACAGGAAGGAC-3' (SEQ ID NO:118) and Monarch-1 pyr-NBD reverse 5'-ATCTCCCTGCAGTTGATGTAGAAG-3' (SEQ ID NO:119).

5' RACE. 5' RACE was performed using two gene-specific primers following the manufacturer's protocol (Roche, Indianapolis, IN). The gene-specific primers were: SP1-5'-CGTCTGGCTCAAAGAGGGTCTCTATC-3' (SEQ ID NO:120) and SP-2-5'-CTGCGGACATAGTCCCTGTAGGTTTC-3' (SEQ ID NO:121). The longest clone was selected as the 5' start of the Monarch-1 mRNA.

Stimulation of Cells with Bacterial Components. Primary human adherent cells, granulocytes or the Thp-1 monocytic cell line were stimulated for the indicated timepoints with lipopolysaccharide (LPS), unextracted LPS, or phenol-extracted LPS at 200 ng/ml as indicated. Lipoteichoic acid (LTA)

was used at 1 µg/ml and Pam3Cys at 100 ng/ml. Cells were harvested at the indicated timepoints and analyzed for Monarch-1 expression using real-time PCR. For tolerance studies, Thp-1 cells were initially stimulated at 200 ng/ml for 18 hours, washed and rested for 1 hour, and then restimulated with 1 µg/ml LPS for 6 hours.

AFFYMETRIX® Analysis. Total RNA from pcDNA-HA and HA-Monarch-1 HeLa stable clones was prepared using RNEASY® Mini columns (QIAGEN®, Valencia, CA). Ten µg of RNA were reverse-transcribed using Superscript II (STRATAGENE®, La Jolla, CA), labeled using the Enzo Bioarray High Yield RNA Transcript Labeling Kit (Enzo Diagnostics, Inc., New York, NY), and analyzed on HG U133A chips according to the AFFYMETRIX® technical manual (<http://www.affymetrix.com>). Sample quality was assessed by examining 3'-5' intensity ratios of control genes. Arrays were scaled to an average intensity of 2500, and expression data analyzed using GENESPRING® software (Silicon Genetics, Redwood City, CA). Altered genes were identified by filtering for increase or decrease in all three Monarch-1-expressing clones compared to their respective control clone of 1.4-fold or more, with a minimum hybridization signal of 500 in the higher expressed sample. P values were determined using AFFYMETRIX® Suite 5.0.

Cytometric Fluorometric Analysis of HLA. Flow cytometry was performed using well-known methods (Martin, *et al.* (1997) *Immunity* 6:591). FITC-conjugated human pan-reactive HLA antibody (CalTag, Burlingame, CA) and control FITC mouse IgG2a κ isotype antibody (Pharmingen, San Diego, CA) were utilized.

Small Interference RNA (siRNA) Construction and Transfection. Wild-type and mutant human Monarch-1 short hairpin RNAs were stably expressed in the human U937 or THP-1 monocyte cell line by transfection of plasmids containing short hairpin RNA transcription cassettes followed by clonal selection in puromycin using well-known methods. The target sequence was:

GTCCATGCTGGCACACAAG (SEQ ID NO:122) and the mutant sequence was: GTCCATGCTAACACACAAG (SEQ ID NO:123).

Cytometric Bead Assay (CBA). Stable THP-1 clones from wild-type and mutant human Monarch-1 siRNA were stimulated with phenol purified LPS for 48 hours. Supernatants were subjected to an inflammatory cytokine CBA panel following the manufacturer's instructions (BD Pharmingen, San Diego, CA). Flow cytometric analysis was performed using standard methodologies.

Cell Culture, Plasmids and Antibodies. 293T cells (Gene Hunter) were maintained in DMEM supplemented with 10% fetal calf serum, 100 mM penicillin, and 100 mM streptomycin and cultured at 37°C and 5% CO₂. cDNA expressing human Monarch-1, TRAF6, MyD88, and IRAK1 were cloned into a pcDNA3-based vector in frame with a double 5' HA tag sequence. FLAG®-tagged Monarch-1 and CIAS1 were amplified by PCR to contain an N-terminal FLAG®-tag and cloned into pcDNA3.1 (INVITROGEN™, Carlsbad, CA). The pCMV-Sport6 vector containing NF-κB Inducing Kinase cDNA was obtained from the Mammalian Genome Collection (Image ID# 5497185). Anti-Flag M2-agarose was obtained from Sigma (St. Louis, MO) and the anti-HA antibody 12C5 was obtained from Roche (Indianapolis, IN). The anti-NIK antibody (H-248) was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Transfections and Immunoprecipitations. 2.5 million 293T cells were seeded in 10-cm cell culture plates. Eighteen to twenty-four hours later, 3 µg of each of the indicated plasmids was transfected using FUGENE6™ (Roche, Indianapolis, IN) at a 3:1 FUGENE6™ to DNA ratio and the cells were incubated an additional 18 to 24 hours. Cells were lysed in 0.5% NP-40, 150 mM NaCl, 50 mM Tris pH 8.0, 160 mM EDTA, 50 mM NaF, 10 mM sodium phosphate supplemented with protease inhibitor cocktail (Roche, Indianapolis, IN). Nuclei were removed and the resulting supernatant was pre-cleared with mouse IgG-conjugated agarose (Sigma, St. Louis) for 1 hour. FLAG®-tagged proteins were subsequently immunoprecipitated overnight with 35 µl M2-agarose equilibrated in lysis buffer. Beads were washed four times in lysis

buffer and proteins eluted in sample buffer (20% Glycerol, 4% SDS, 130 mM Tris pH 6.8, 20 mM DTT) for western blot analysis.

Western Blot Analysis. Proteins were separated by polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (BIO-RAD®, Hercules, CA). Membranes were blocked in 1% BSA in Tris-buffered saline-Tween (TBS-T; 10 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween-20) for one hour then incubated with the indicated primary antibody overnight. Membranes were washed five times in TBS-T and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody for 30 minutes. Following five additional washes in TBS-T, proteins were visualized by Enhanced Chemiluminescence (Pierce Chemical Co., Rockland, IL).

Two Dimensional Gel Electrophoresis. Ten 10-cm plates were transfected with FLAG®-tagged Monarch-1 or empty vector as described. Following immunoprecipitation, the beads were combined into one sample and protein complexes were eluted with rehydration buffer (8 M urea, 2 M thiourea, 2% CHAPS, 20 mM DTT, 0.7% immobilized pH gradient (IPG) buffer ampholytes (AMERSHAM™, Piscataway, NJ). Proteins were separated based on isoelectric point in the first dimension using IPG strips (AMERSHAM™, Piscataway, NJ) for 57,700 volt hours. The IPG strips were transferred to pre-cast 10% polyacrylamide gels (BIO-RAD®, Hercules, CA) and the proteins separated by molecular weight in the second dimension. Protein spots were visualized by silver staining (Blum, *et al.* (1987) *Electrophoresis* 8:93-99). Protein profiles were compared between empty vector-transfected and FLAG®-Monarch-1-transfected samples and spots unique to FLAG®-Monarch-1-transfected samples were picked for mass spectrometry analysis. Excised protein spots were trypsin digested and processed for MALDI-MS. Protein identities were determined by comparing peptide mass fingerprints to the NCBI, SwissProt, and TrEMBL protein databases using the following software: Mascot (Matrix Sciences, London, UK), Profound (University of California-San Francisco, CA), and PeptIdent (EMBL).

RNA Interference Vector for in vivo Knockdown in Mice. An RNA interference vector containing the siRNA provided herein is inserted into a plasmid wherein transcription of the Monarch-1 specific siRNA is under the control of a pol III promoter. Using this plasmid, the siRNA to Monarch-1 is expressed in hematopoietic stem cells. Alternatively, the siRNA is inserted into a targeting vector and ES cells harboring this vector are generated and screened for homologous recombination of the Monarch-1 gene. ES cells with a Monarch-1 gene knockout are used to generate a Monarch-1 knockout mouse.

Experimental Results

Identification of the Human Monarch-1 cDNA. Genes of novel NBD/LRR proteins with structural similarities to CIITA were identified in searches of the published Celera and the NCBI human genome databases (EXAMPLE 1). One predicted gene, Monarch-1 was cloned by RT-PCR using primer pairs specific for nucleic acid sequences encoding both the identified N- and C-terminal regions of the protein. The 5' end of the longest clone was isolated using RACE-PCR of cDNA from U937 cells. The full-length human cDNA, corresponding to accession number AY116204 (SEQ ID NO:1; **Figures 6A-B**), was 3731 bp long with a 220 bp 5'-UTR, a 323 bp 3'-UTR and a 3189 bp open reading frame. Monarch-1 is located on human chromosome 19q13.4. Comparison with known mRNAs in the database revealed the 3' one-third of this gene was previously identified as RNO2 (Shami, *et al.* (2001) *Br. J. Haematol.* 112:138). The Monarch-1 cDNA, contained in ten exons, encoded a predicted protein of 1063 amino acid residues (SEQ ID NO:2; **Figure 6C**) with a predicted molecular mass of 118 kDa. Multiple Monarch-1 splice forms were identified by conducting RT-PCR on PBMC total RNA using primers spanning the end of the NBD through the C-terminal LRR region of Monarch-1. At least four splice forms of the Monarch-1 LRR region were evident. Sequence analysis of the four prominent bands showed that these novel splice forms corresponded to differential splicing of the LRR and were identified as accession number AY116205 (SEQ ID NO:3; **Figures 6D-E**),

AY116206 (SEQ ID NO:5; **Figure 6G-H**), and AY116207 (SEQ ID NO:7; **Figure 6J-K**). The full-length Monarch-1 mRNA contains 10 exons and encodes isoform I (SEQ ID NO:2), while nucleic acids encoding isoform II (SEQ ID NO:4; **Figure 6F**) lack exon 9, nucleic acids encoding isoform III (SEQ ID NO:6; **Figure 6I**) lack exons 7 and 8, and nucleic acids encoding isoform IV (SEQ ID NO:8; **Figure 6L**) lack exons 7 through 9. Analysis of Monarch-1 using RT-PCR with primers specific for nucleic acids encoding the N-terminal region indicated that alternative N-terminal splice forms do not exist.

Expression of Monarch-1 is Predominantly in Myeloid-Monocytic Cells.

RT-PCR showed expression in U937 and HL-60 cells but not T/B or non-hematopoietic cell lines. Monarch-1 expression was assessed in PBMC subpopulations by RT-PCR and showed expression in dendritic cells, monocytes and granulocytes. A faint band was detected in the lymphocyte preparation, however this may have been due to contamination as these same preparations showed a faint band for the myeloid genes, CD14 and CD15. To more definitively compare Monarch-1 expression among the myeloid-monocytic cells, real-time PCR analysis was employed using forward primer 5'-AGAGGACCTGGTGAGGGATAC-3' (SEQ ID NO:124), reverse primer 5'-CTTCCAGAAGGCATGTTGAC-3' (SEQ ID NO:125) and probe 5'-CCCGTCCTCACTTGGGAACCA-3' (SEQ ID NO:126). High levels of Monarch-1 were detected in granulocytes, with lower expression observed in monocytes (**Figure 7A**). An increase in Monarch-1 expression was observed in monocytes in response to DETA-NO (an activator of nitric oxide) consistent with previous findings of nitric oxide induction of RNO2 mRNA expression (Shami, *et al.* (2001) *supra*). In contrast, TFN- α , IFN γ , or a combination of the two decreased Monarch-1 expression in a time-dependent fashion (**Figure 7B**).

Identification of Monarch-1 Regulated Genes by DNA Microarray Analysis. The downstream effects of increased Monarch-1 were determined using AFFYMETRIX® DNA array analysis to compare gene profiles in the

presence or absence of Monarch-1. Stable clones expressing Monarch-1 were made in the HeLa cell line because this cell line does not express Monarch-1 (**Figure 8**). Two sets of stable expressing Monarch-1 clones were independently produced on different days by transfection of HeLa cells with either the empty vector control, pcDNA, or with a pcDNA-HA-tagged Monarch-1 expression vector and selected for neomycin resistance. The first experiment resulted in two Monarch-1-containing clones, clone A with lower Monarch-1 expression and clone B with higher expression. The second experiment resulted in one clone, C, with intermediate expression. Analysis of the Monarch-1 expression level in different RNA preparations of these clones relative to total primary human PBMCs indicated that the clones expressed lower levels of Monarch-1 than PBMCs. Thus, changes detected in Monarch-1 expressing lines are relevant and not due to the overexpression of Monarch-1. Clones with a higher Monarch-1 level were not obtained.

DNA microarray analysis was performed for control and Monarch-1 expressing stable clones using AFFYMETRIX® chips comprising approximately 22,000 gene sequences. The most prominent change was a cluster of nine MHC class I-related sequences, including HLA-B (three sequences), HLA-C (two sequences), HLA-F (one sequence), HLA-G (two sequences), and the proteosomal subunit LMP7 required for processing of class I peptides (**Table 3**, samples with "x"). Multiple appearances of HLA-B, C, and G indicated the validity of these findings. Regulation of MHC-II genes by Monarch-1 was not observed. Expression patterns of all HLA genes was further analyzed to assess if additional MHC-I genes may be modulated by Monarch-1 but were not included due to the stringent cutoff standards used for filtering. All classical MHC-I (HLA-A, B and C) and nonclassical MHC-I genes (HLA-E, F and G) were upregulated by Monarch-1.

TABLE 3

Accession #		Clone A	Clone B	Clone C	Gene Name
AI923492		1.3 *	1.6 *	2.0 *	HLA-A
AA573862		1.4 *	1.8 *	2.4 *	HLA-A
L07950	x	1.7 *	2.7 *	2.6 *	HLA-B
D83043	x	1.8 *	2.6 *	4.0 *	HLA-B
L42024	x	1.5 *	2.3 *	3.1 *	HLA-B
AK024836	x	1.8 *	2.2 *	3.1 *	HLA-C
U62824		1.3 *	2.0 *	2.1 *	HLA-C
M12679	x	1.4 *	1.8 *	3.7 *	HLA-C
BC004489	x	1.5 *	1.9 *	3.9 *	HLA-C
M31183		2.4 *	2.9 *	1.1 +	HLA-E
NM_005516		1.6 *	2.0 *	1.1 *	HLA-E
NM_018950		1.6 *	2.0 *	1.9 *	HLA-F
AW514210	x	1.4 *	2.0 *	1.5 *	HLA-F
AF226990		2.0 *	2.8 *	3.8 *	HLA-G
M90686	x	2.3 *	2.7 *	2.1 *	HLA-G
M90684	x	1.3 *	1.8 *	2.5 *	HLA-G
M80469	x	1.1	1.6 +	2.5 *	HLA-J
U17496	x	1.5 *	2.9 *	4.1 *	LMP7

Fold induction for each clone was calculated relative to its control clone. "x" indicate genes identified in the original analysis. P values were determined using AFFYMETRIX® Suite 5.0 (* = $p < 0.01$, + = $p < 0.05$).

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Changes in expression levels were quantified using real-time PCR analysis of total RNA isolated from A, B and C stable clones. The levels of HLA-B, HLA-G and LMP7 mRNA were enhanced in the Monarch-1 stable clones compared to controls (**Figure 9A**). FACS analysis further confirmed upregulation of MHC-I antigen (**Figure 9B**). To discern the involvement of transcriptional or posttranscriptional mechanisms, a Monarch-1 expression plasmid (or a control plasmid) was transiently co-transfected with a luciferase reporter driven by 220 bp of the HLA-B promoter (Gobin and van den Elsen (1999) *Semin. Cancer Biol.* 9:55) in HeLa cells (**Figure 9C**). Monarch-1 enhanced the HLA-B promoter >25-fold. This enhanced activity over that seen for mRNA and protein levels may be due to transient transfection resulting in higher than physiological levels of Monarch-1.

As the level of expression in the stable clones was less than that in primary blood cells, the regulation of MHC genes was performed in a more relevant system. siRNA technology was used to reduce endogenous Monarch-1 expression levels in U937 cells, which express Monarch-1 and

ASC (Masumoto, *et al.* (1999) *J. Biol. Chem.* 274:33835). A vector containing an siRNA specific for Monarch-1 or a mutant siRNA with two mutated nucleotides was introduced into U937 cells. The bulk culture which should have a mixture of cells containing or lacking Monarch-1 specific siRNA showed a decrease of overall Monarch-1 expression compared to cells with control siRNA (**Figure 10A**). Clones were then isolated under selectable conditions. Monarch-1 expression was significantly decreased in Monarch siRNA clones, but not in the controls (**Figure 10B**, top panel). The levels of Monarch-1, HLA-B and HLA-G mRNA were correspondingly decreased in the presence of Monarch-1-specific siRNAs but not siRNA controls (**Figure 10B**, two lower panels). These data indicate that Monarch-1 controls both classical and nonclassical MHC-I genes in a physiologically relevant cell type and may function as a novel global inducer of MHC-I.

While IFN γ and TNF α , known inducers of class I molecules, decreased Monarch-1 expression, no significant alteration of MHC class I HLA-G gene expression was observed at these timepoints. At later time points, HLA-G expression was enhanced by these two cytokines. This indicates that Monarch-1 may not play a major role in the induction of MHC-I by TNF- α and IFN- γ .

Expression of Human Monarch-1. Human primary adherent cell populations or granulocytes were isolated from human peripheral blood buffy coats using a FICOLL® gradient. The adherent population or granulocytes were subsequently exposed to either LPS or peptidoglycan (PGN) for 1 hour. RNA was harvested followed by real-time PCR analysis for Monarch-1 mRNA expression using primers C-term forward 5'-CAGAAGGACATCAACTGTGAGAG-3' (SEQ ID NO:127) and C-term reverse 5'-GCTCTAGACAGCAGATAGGACCATTCAGCAG-3' (SEQ ID NO:128). A down-regulation of Monarch-1 mRNA expression levels by PGN (TLR2 ligand) and LPS (TLR4 ligand) was observed 1 hour after stimulation in both granulocytes and monocytes (**Figure 11**) and the Thp-1 cell line. As some commercially available reagents may be contaminated with endotoxin, granulocytes were stimulated with phenol-purified LPS and the synthetic

TLR2 agonist Pam3Cys. The observed down-regulation of Monarch-1 in the phenol-purified LPS and Pam3Cys-treated cells confirmed that Monarch-1 expression was down-regulated by exposure of cells to TLR2 and TLR4 agonists (**Figure 11**). Data from at least four experiments indicated that the TLR2 agonist, LTA, did not cause a down-regulation of Monarch-1 expression. It is known that LTA and Pam3Cys signal through slightly different downstream pathways, and these data indicated that Monarch-1 down-regulation may be specific to certain bacterial components. Monarch-1 down-regulated upon exposure of the human Thp-1 cell line to LPS was shown, however, the mRNA for this molecule returns 18 hours after LPS stimulation in Thp-1 cells, and was not further reduced upon a second LPS stimulation (**Figure 12**). This second LPS stimulation was typically applied to measure LPS tolerance or endotoxin tolerance, a state of LPS non-responsiveness following an initial LPS stimulation. LPS tolerance may reflect events that occur in septic shock survivors who exhibit suppressed monocytic and inflammatory responses to subsequent LPS. TLR pathway mediators such as IRAK and MyD88 are defective during LPS tolerance. As Monarch-1 expression re-appears during this "LPS"-tolerant phase, the effect of Monarch-1 on TLR-induced downstream signals was examined.

Regulation of TLR-Induced NF- κ B Activity by Monarch-1. Members of the CATERPILLER family of proteins have been found to be critical for apoptosis, immune and inflammatory diseases. Thus, regulation by Monarch-1 of the TLR signaling molecule induced NF- κ B activation was examined. HEK293T cells were transiently transfected with a Monarch-1-encoding plasmid together with an NF- κ B-dependent luciferase reporter. NF- κ B activity was induced by co-transfection with TLR signaling pathway molecules including MyD88, IRAK, TRAF6 and TRAF2. Under these experimental conditions, Monarch-1 appeared to inhibit NF- κ B induction by TLR signaling molecules (**Figure 13**). These data indicate that Monarch-1 is a negative regulator of NF- κ B activity when introduced into epithelial cell lines.

Human Monarch-1 Expression in Lung BAL. Bronchiolar lavage samples were obtained from normal human subjects and lung transplant patients. Real-time PCR analysis for Monarch-1 mRNA expression indicated that Monarch-1 expression was down-regulated in BAL samples from
5 transplant patients (**Figure 14**).

Human Monarch-1 and Cytokine Expression. Stable Thp-1 clones expressing mutant siRNA Monarch-1 oligo (designated by M followed by clone number in **Figure 15**) and six clones expressing wild-type siRNA oligo
10 targeting Monarch-1 expression (designated by clone number) were either unstimulated (designated by C) or stimulated with LPS (designated by L) for 24 hours. RNA was harvested and IL-10 expression was examined by real-time PCR. All clones expressing a Monarch-1 siRNA oligo expressed dramatically lower levels of IL-10 than clones expressing the mutant oligo. As
15 Monarch-1 expression returned to normal levels at 24 hours after LPS stimulation and Monarch-1 was not down-regulated in LPS tolerant cells (**Figure 12**), these data indicate that Monarch-1 is a positive regulator of the anti-inflammatory IL-10 cytokine. IL-10 is both an immunosuppressive molecule, as well as a molecule that diverts T cell responses to a T-helper 2
20 response, leading to an anti-parasitic response, allergic response, asthma response and antibody response. Further, stable Thp-1 clones expressing mutant siRNA Monarch-1 oligo and six clones expressing wild-type siRNA oligo targeting Monarch-1 expression were either unstimulated or stimulated with LPS for 3 hours. RNA was harvested and TNF α expression was
25 examined by real-time PCR. All clones expressing a Monarch-1 siRNA oligo expressed lower levels of TNF α than clones expressing the mutant oligo (**Figure 16**).

A more global analysis of cytokine gene expression was conducted. Stable Thp-1 clones expressing mutant siRNA Monarch-1 oligo (designated
30 by M followed by clone number) and six clones expressing wild-type siRNA oligo targeting Monarch-1 expression (designated by clone number) were either unstimulated (designated by C) or stimulated with LPS (designated by L) for 48 hours. Supernatants were isolated and a Cytometric Bead Assay

(CBA) was performed (Figure 17). All clones expressing a Monarch-1 siRNA oligo expressed lower levels of IL-6, IL-1b, and IL-10 than clones expressing the mutant oligo. In contrast, basal and stimulated levels of IL-8 were similar to the mutant control clones.

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Proteomic Analysis of Monarch-1 Interacting Proteins. Interacting partners of Monarch-1 were identified by transfecting 293T cells with FLAG®-tagged Monarch-1 or pcDNA control vector. After 24 hours, the cells were lysed and protein complexes were immunoprecipitated with anti-FLAG®
10 antibodies. Proteins were solubilized and separated by two-dimensional gel electrophoresis. Individual protein spots were visualized by silver staining and those found to be unique to Monarch-1-transfected precipitates were analyzed by matrix-assisted laser desorption/ionization mass spectrometry mass spectrometer (MALDI-MS). Proteins identities were determined by comparing
15 peptide mass fingerprints to the NCBI, SwissProt, and TrEMBL protein databases. Proteins associating with Monarch-1 having the highest significance scores were identified as β and α -tubulin, vimentin, hsp-70 family members. CARD10 and TNIK were also identified with lower significant scores. The interaction between Monarch-1 and vimentin may indicate that
20 Monarch-1 binds to vimentin intracellularly to inhibit the bactericidal activity of secreted vimentin (Mor-Vaknin, *et al.* (2003) *Nature Cell Biol.* 5:59-63). Further the interaction between Monarch-1 and CARD10 may indicate that Monarch-1 inhibits NF-kB activation by interfering with CARD10.

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Monarch-1 Associates with TRAF6 but not MyD88 or IRAK1. In addition to the proteins identified by MALDI-MS, 293T cells were transfected with Monarch-1 cDNA and one of the following: HA-MyD88, HA-TRAF6, or HA-IRAK1. Twenty-four hours later the cells were lysed and Monarch-1-containing protein complexes were immunoprecipitated with anti-FLAG® M2-
30 agarose beads overnight. The beads were washed in lysis buffer and precipitated proteins were separated by polyacrylamide gel electrophoresis. Western blot analysis was performed using the anti-HA antibody 12C5

(Roche, Indianapolis, IN) and anti-mouse conjugated to HRP. Of these proteins, only TRAF6 binds Monarch-1.

Monarch-1 Associates with NF- κ B Inducing kinase, NIK. To further
5 determine the molecular mechanism by which Monarch-1 may interface with
the NF- κ B pathway, 293T cells were transfected with plasmids containing
Monarch-1 and NIK. After 24 hours, the cells were lysed and Monarch-1-
containing protein complexes were immunoprecipitated with anti-FLAG® M2
antibody. Western blot analysis was performed using the anti-NIK antibody
10 H-248 and anti-mouse conjugated to HRP. The results indicate that Monarch-
1 also interacts with NIK.

Monarch1 Associates with CIAS1. 293T cells were transfected with
plasmids containing the Monarch-1 and CIAS1 genes (also a CATERPILLER
15 family member). After 24 hours, the cells were lysed and CIAS1-containing
protein complexes were immunoprecipitated with anti-FLAG® M2 antibody.
Western blot analysis was performed using the anti-HA antibody and anti-
mouse conjugated to HRP. An association between CIAS1 and Monarch-1
was observed and enhanced in the absence of LRR regions 7-9 of Monarch-
20 1. In contrast, the association was weakened in the absence of the pyrin
domain of Monarch-1.

Predicted Mouse Monarch-1 mRNA and Protein Sequence. A predicted
mouse Monarch-1 sequence was identified corresponding to NCBI database
25 Accession Number XM_142563 (SEQ ID NO:9; incorporated by reference
herein in its entirety). The predicted size of the mouse Monarch-1 mRNA was
3102 bp (**Figure 18A-B**) which encodes a 1035 amino acid residue protein
(SEQ ID NO:10; **Figure 18C**). The predicted mouse Monarch-1 gene
contains 9 exons; however, nucleic acid sequences corresponding to exon 2
30 of the human Monarch-1 gene were not predicted. Thus, the mouse Monarch-
1 gene may contain 10 exons similar to its human homolog. Moreover, the
mouse Monarch-1 protein had structural characteristics similar to human
Monarch-1, each contained an N-terminal Pyrin domain, a nucleotide-binding

domain (NBD) and C-terminal leucine-rich repeats (LRR). The human and mouse Monarch-1 share 82% nucleotide sequence homology.

Expression of Mouse Monarch-1. Real-Time PCR analysis showed expression of mouse Monarch-1 in primary PBMCs and bone marrow (**Figure 19**). Low levels of Monarch-1 were detected in testis, spleen and liver tissues from a perfused mouse (designated by -perf) but not in other perfused tissues tested. Perfusion was necessary to eliminate contaminating blood cells. Monarch-1 expression was detected in non-perfused tissues at a slightly higher level indicating blood contamination. Monarch-1 expression was not detected in cell lines tested, although a negligible level was detected in B16-F10 fibroblast cells. Mouse Monarch-1 expression was not detected in Brewers Thioglycolate mouse peritoneal macrophages, even upon exposure to LPS. Mouse Monarch-1 expression was detected in CD11b+/Gr-1+ myeloid suppressor cells isolated from BALB/c mice with large primary mammary carcinomas (tumor-bearing) and mice with metastasis wherein primary mammary tumors had been surgically removed (non-tumor-bearing) (**Figure 20**). The expression level of Monarch-1 was higher in myeloid suppressor cells from tumor-bearing mice than in non-tumor-bearing mice.

EXAMPLE 3

Cloning, Characterization and Functional Analysis of CATERPILLER 11.2

This example describes the characteristics of the human CATERPILLER 11.2 gene. The cloned human CATERPILLER 11.2 nucleic acid and protein sequences are set forth as SEQ ID NO:13 and SEQ ID NO:14, respectively. It has now been found that CATERPILLER 11.2 reduces the function of NF- κ B. CATERPILLER 11.2 expression is primarily found in hematopoietic cell lines. The reduction of NF- κ B function by CATERPILLER 11.2 indicates that CATERPILLER 11.2 is important in the control of immunity, gene expression and cell survival. In addition, CATERPILLER 11.2 also suppresses the expression of the class II Major Histocompatibility Complex

(MHC-II) promoter. Proper MHC-II expression is important for immune recognition to elicit T cell responses against all pathogens and antigens.

Materials and Methods

5 *Detection of CATERPILLER 11.2 Expression by PCR.* RNA samples from various sources, including normal peripheral blood and human cell lines, were prepared using commercially available reagents following the manufacturer's instructions. 50 ng to 1 µg of RNA was reverse transcribed with CATERPILLER 11.2-specific primers using the QIAGEN® One-Step RT-PCR kit (QIAGEN®, Valencia, CA).

15 *Cloning of CATERPILLER 11.2.* Primers pairs corresponding to the 5' and 3' ends of the predicted gene were used to amplify two fragments of the CATERPILLER 11.2 gene and were as follows. 5'-AAC TTT GCC TTT GAA GAA CCT GAG-3' (SEQ ID NO:129) at nucleotide position 793; 5'-ACA TGA AGG TGG GYG AAC ACA TAG-3' (SEQ ID NO:130) at position 1448; 5'-ATG GCA GAT TCA TCA TCA TCA TCT TC-3' (SEQ ID NO:131) at nucleotide position 1; and 5'-TCA CCC GAG CCT CTG AAT GTT ACA G-3' (SEQ ID NO:132) at nucleotide position 2808. Resulting PCR products were cloned into the TOPO® TA cloning vector and the cloned sequence was verified. Full-length FLAG®-tagged CATERPILLER 11.2 was obtained by transferring the two fragments into pcDNA3 and adding a 5' FLAG® epitope tag sequence.

25 *Transfection and Reporter Assays.* HeLa cells were transfected using the FUGENE6™ transfection reagent. 2×10^5 cells received 1.0 µg of FLAG®-11.2 or empty vector together with 0.5 µg of reporter plasmid (3X NFκBLuc, AP1Luc, or DRLuc) and either 100 ng of activator plasmid (NF-κB p65, c-jun, or CIITA) or empty vector. Eighteen hours post-transfection, luciferase assays were performed using standard protocols.

30 *RNA interference.* The follow sequence was designed to inhibit expression of CATERPILLER 11.2 in cells: 3'-GAT CCC CGA AGA GAT CAA

CTG GTC GGT TCA AGA GAC CGA CCA GTT GAT CTC TTC TTT TTG
GAA AGG GCT TCT CTA GTT GAC CAG CCA CGT TCT CTG GCT GGT
CAA CTA GAG AAG AAA AAC CTT TAG CT-3' (SEQ ID NO:133). This
sequence is cloned into a plasmid and stably expressed in a human cell line
5 to interfere with the expression of CATERPILLER 11.2 *in vivo*.

Experimental Results

Genomic Organization and Sequence of CATERPILLER 11.2. The
CATERPILLER 11.2 gene resides at 11p15 on human chromosome 11 and
10 contains as many as 8 exons (**Figure 22**) based on both bioinformatics
predictions and obtained sequence data. Using the predicted sequence (SEQ
ID NO:11; **Figure 21A-B**) encoding the CATERPILLER 11.2 protein (SEQ ID
NO:12; **Figure 21C**), assembly of PCR products from the B cell line Raji
yielded an approximately 2.8 kilobase pair (kb) insert containing both the
15 putative initiator codon (ATG) and an in-frame stop codon that precedes a
canonical poly-adenylation site. The DNA sequence of this fragment (SEQ ID
NO:13) and the deduced protein sequence (SEQ ID NO:14) are shown in
Figures 21D-E and **Figure 21F**, respectively. This insert was cloned into the
pcDNA3 expression vector (INVITROGEN™, Carlsbad, CA) and was tagged
20 with the FLAG® epitope for detection with anti-FLAG® monoclonal antibodies.

Expression of CATERPILLER 11.2. CATERPILLER 11.2 was
expressed by some common cell lines, but did not appear to be expressed in
primary hematopoietic cells. CATERPILLER 11.2 mRNA transcripts were
25 detected in a number of human B cell lines indicating that CATERPILLER
11.2 may be expressed in later stages of B cell development. While other
CATERPILLER genes (e.g., CIAS1) were detected in *in vitro* matured
dendritic cells (DCs), 11.2 mRNA was not detected.

Transcriptional Inhibition Functions of CATERPILLER 11.2.
30 Expression of CATERPILLER 11.2 inhibited activation of the 3XNFκB
luciferase reporter by transfected NF-κB p65 (**Figure 23A**), but did not inhibit
activation of the AP1 luciferase reporter by transfected c-jun (**Figure 23B**).

This indicates that CATERPILLER 11.2 acts to inhibit p65 activity either directly, through binding to p65, or indirectly by interfering with the ability of p65 to transit effectively to the nucleus or bind DNA. Alternatively, 11.2 may promote the inactivation of p65 through such mechanisms as p65 degradation or blockade of modifications needed for p65 activity. CATERPILLER 11.2 also inhibited CIITA-mediated HLA-DR transcription as evidenced by its effects on the HLA-DR promoter (**Figure 24**). This reporter is not believed to be sensitive to NF- κ B, thus other factors may be responsible for the observed effect. For example, the formation of hetero-oligomers with CIITA itself may be responsible as they are related molecules and CIITA is known to self-associate. Such an interaction may interfere with the ability of CIITA to enter the nucleus or prevent proper association with transcription factors bound to the HLA-DR promoter.

EXAMPLE 4

Cloning and Characterization of CATERPILLER 11.3

This example describes the characteristics of the cloned human CATERPILLER 11.3 gene (SEQ ID NO:17; **Figure 25C-D**) and a protein encoded thereby (SEQ ID NO:18; **Figure 25E**) as well as a CATERPILLER 11.3 splice variant (SEQ ID NO:19; **Figure 25F**) and a protein encoded thereby (SEQ ID NO:20; **Figure 25G**).

Materials and Methods

Cloning of Full-Length Human 11.3. Primers designed for detecting expression of CATERPILLER 11.3 were originally developed from the predicted sequence of CATERPILLER 11.3 (see EXAMPLE 1 and **Figure 3**; nucleotide sequence [SEQ ID NO:15; **Figure 25A**]; amino acid sequence [SEQ ID NO:16; **Figure 15B**]). To obtain the full-length open reading frame of human CATERPILLER 11.3, total and polyA⁺ RNA were isolated from a Jurkat T cell line. PolyA⁺ mRNA was isolated from the total RNA using the OLIGOTEX® mRNA Mini Kit (QIAGEN® Inc., Valencia, CA). The complete 5' sequence of human CATERPILLER 11.3 was cloned using the 5'RACE kit (Roche, Indianapolis, IN). Two overlapping sequences were cloned for the

remaining portion of human CATERPILLER 11.3 by RT-PCR using PFUTURBO® polymerase (STRATAGENE®, Inc., La Jolla, CA). Using these three separate clones, splice overlap extension was performed using *Taq* polymerase (INVITROGEN™ Life Technologies, Carlsbad, CA). The resulting full-length clone and splice variant were ligated into a pcDNA3.1 expression vector containing tandem HA epitopes. In addition, a FLAG® epitope was added to the 5' end of human CATERPILLER 11.3 by RT-PCR and ligated into pcDNA3.1. All cloned products were sequenced for verification.

Human 11.3 Expression. Total RNA was isolated from various sources, including transformed human cell lines and normal peripheral blood, using the SV total RNA isolation system (PROMEGA™, Madison, WI). Following RNA isolation, 1 µg RNA was reverse-transcribed and PCR was performed using primers specific for the NBD to determine the expression pattern of CATERPILLER 11.3. In addition, real-time PCR primers were designed for subsequent quantitative expression analysis using the TAQMAN® sequence detection system (Applied Biosystems, Foster City, CA)

Luciferase Reporter Gene Assays. HEK293T cells were plated at 1 x 10⁴ cells/well in 96-well plates and transfected the following day using FUGENE™ transfection reagent (Roche, Indianapolis, IN) following the manufacturer's recommended protocol. For NF-κB or AP-1 reporter assays, cells were transfected with 50 ng of NF-κB-luc and various amounts agonists (MyD88, NIK, Traf6, IRAK1), maintaining the total amount of DNA constant using pcDNA3 empty vector. The p53 reporter assays were performed with 50 ng of p53-luc reporter plasmid, 200 ng of p53 expression vector and 400 ng of CATERPILLER 11.3 expression plasmid. Cells were harvested 24 hours after transfection and assayed for luciferase activity following standard procedures with the equal amounts of protein as determined by the Bradford protein assay (BIO-RAD®, Hercules, CA).

Experimental Results

Full-Length Coding Sequence of Human CATERPILLER 11.3.

The CATERPILLER 11.3 gene resides at 11q23 on human chromosome 11 and contains as many as 9 exons (**Figure 26**) based on both bioinformatics

5 predictions and sequence data obtained from cloning CATERPILLER 11.3.

Assembly of PCR products from the T cell line Jurkat yielded an approximately 3.6 kilobase pair (kb) insert containing both the initiator codon (ATG) and an in-frame stop codon that precedes a 3'-UTR and poly-

10 adenylation site. The DNA sequence of this fragment (SEQ ID NO:17) and the deduced protein sequence (SEQ ID NO:18) are shown in **Figures 25C-D** and **Figure 25E**, respectively. This insert was cloned into the pcDNA3

expression vector (INVITROGEN™, Carlsbad, CA) and was tagged with the FLAG® and HA epitope for detection with monoclonal antibodies.

15 Additionally, a splice variant of CATERPILLER 11.3 was cloned and sequenced with nucleotide (SEQ ID NO:18) and deduced amino acid (SEQ ID NO:19) sequences shown in **Figure 25F and 25G**, respectively.

Expression of Human CATERPILLER 11.3. It has now been shown that human CATERPILLER 11.3 is expressed in a variety of cells.

20 CATERPILLER 11.3 expression was found in T cell, B cell, and myeloid cell lines. In addition, CATERPILLER 11.3 was expressed by primary T-cells with reduced expression in the presence of PHA. Expression in HL-60 cells was also abrogated in response to PMA stimulation. Human CATERPILLER 11.3 did not appear to be expressed by cells of epithelial origin. In addition, mouse

25 CATERPILLER 11.3 was expressed in resting murine CD4+CD25+ T regulatory cells at 10-fold higher levels than CD4+CD25- T cells, indicating that CATERPILLER 11.3 may control T regulatory cell function. T regulatory cells are typically associated with a suppressive phenotype. Also, mouse CATERPILLER 11.3 expression in murine T regulatory cells isolated from
30 mice on a B6 background was 10-fold higher than cells harvested from the autoimmune sensitive SLJ background.

Transcriptional Inhibitory Functions of CATERPILLER 11.3.

Transfection of CATERPILLER 11.3 inhibited the activation of the NF- κ B luciferase reporter by transfected MyD88 (**Figure 27**). MyD88 is an important adapter protein that links members of the toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) superfamily to the downstream activation of nuclear factor- κ B and mitogen-activated protein kinases. Transfection of CATERPILLER 11.3 abrogated activation of NF- κ B luciferase reporter by transfected NIK (NF- κ B Inducing Kinase) (**Figure 28**). In addition to MyD88, NIK has been shown to be an important molecule for NF- κ B signaling. These data indicate that human CATERPILLER 11.3 functions as an inhibitory molecule in the inflammatory signaling pathways leading to activation of NF- κ B. This inhibitory effect may be mediated through interactions between CATERPILLER 11.3 and molecules such as MyD88 and NIK.

EXAMPLE 5

Cloning, Characterization, Expression and Functional Analysis
of CATERPILLER 16.1

This example describes the characteristics of the cloned human CATERPILLER 16.1 gene (SEQ ID NO:23) and the protein encoded thereby (SEQ ID NO:24). Expression of CATERPILLER 16.1 was found in, but not restricted to, cell lines and primary human cells of hemotapoietic origin, including B and T lymphocytes, monocytes and granulocytes. It was found that CATERPILLER 16.1 expression was affected by activation stimuli in Jurkat T cells (human T lymphocyte cell line) and differentiation stimuli in HL-60 cells (human promyleocytic cell line). These results indicate that CATERPILLER 16.1 is involved in both differentiation and activation of certain cell types that may impact host responses to pathogens or the regulation of autoimmune diseases and/or cancer or precancerous conditions.

Materials and Methods

Human CATERPILLER 16.1 Expression. Total RNA was isolated from various sources, including transformed human cell lines and normal peripheral

blood, using the SV total RNA isolation system (PROMEGA™, Madison, WI). Following RNA isolation, 1 µg RNA was reverse-transcribed and PCR was performed using primers specific for CATERPILLER 16.1. In addition, real-time PCR primers were designed for subsequent quantitative expression analysis using the TAQMAN® sequence detection system (Applied Biosystems, Foster City, CA)

Experimental Results

Genomic Organization and Sequence of CATERPILLER 16.1.

CATERPILLAR 16.1 was identified as a predicted novel NBD/LRR protein with structural similarities to CIITA (see EXAMPLE 1). The predicted nucleic acid and protein sequences of CATERPILLER 16.1 are set forth as SEQ ID NO:21 (**Figure 29A-B**) and SEQ ID NO:22 (**Figure 29C**), respectively. It was found that CATERPILLER 16.1 was located on human chromosome 16q13 and was situated between *CTEP* and *CPNE2*. CATERPILLER 16.1 contained as many as 47 exons based on experimental evidence and the transcript was greater than 5500 bp, containing an in-frame stop codon. Two fragments approximately 3000bp in length with 1500 bp overlap were connected by splice overlap extension to clone the predicted gene. The resulting fragment was approximately 4500 bp in length.

A partial 4500 bp sequence of CATERPILLAR 16.1 was cloned from a Raji B cell line and sequenced (**Figure 29D-F**). The protein sequence of the cloned CATERPILLER 16.1 (**Figure 29G**) most closely resembled NOD27. The cloned CATERPILLER 16.1 sequence lacks at least two small exons (underlined in **Figure 30A-D**) present in NOD27 (Accession number AF389420; Dowds, *et al.* (2003) *Biochem. Biophys. Res. Commun.* 302(3)575-580; the disclosures of which are incorporated by reference herein in their entireties). These exons span nucleotides 3248-3327 and 3745-3828 or 3750-3833 (relative to NOD27). In addition, two nonconservative mutations are predicted, P453L and C500R. The origin of the cDNA for NOD27 has not been described, therefore it further differences between NOD27 and CATERPILLER 16.1 may exist due to the differences in origin of cDNA.

5' Region of cloned CATERPILLER 16.1. The 5' region of the cloned CATERPILLER 16.1 sequence did not appear to encode a recognizable pyrin domain. Modeling studies indicated that this region did not contain significant homology with any known structure. 5' rapid amplification of the
5 complimentary ends (RACE) is performed to characterize the 5' region of cloned CATERPILLER 16.1.

Expression of CATERPILLER 16.1. CATERPILLER 16.1 was expressed by many cell lines, including transformed B and T lymphocytes
10 (Raji and Jurkat), and pro-monocytic cell lines (U937, THP-1, HL-60). CATERPILLER 16.1 was also expressed in primary hematopoietic cells, including T and B lymphocytes, monocytes, and granulocytes. CATERPILLER 16.1 expression was decreased significantly in HL-60 cells upon differentiation with phorbol esters, and increased with T cell activation.

15
In vitro Gene Knock Down of CATERPILLER 16.1. RNA interference vectors were constructed to TCTCAGCTTTAAGAGCAGG (SEQ ID NO: 134) and are useful in examining the function of CATERPILLAR 16.1 in Jurkat T cells, Raji B cells, and HL-60 cells.

20
Studies of murine ortholog of CATERPILLER 16.1. Cellular and tissue localization of the murine ortholog of CATERPILLER 16.1, also referred to herein as CATERPILLER m16.1, was determined by real-time PCR. Similar to the human CATERPILLER 16.1, CATERPILLER m16.1 was highly
25 abundant in blood, lymphoid tissues, myeloid and lymphoid cells (see **Figure 31A** and **31B**). The expression of CATERPILLER m16.1 was upregulated by treatment of primary peritoneal macrophage with LPS (see **Figure 31C**). The expression of m16.1 was correlated with several inflammatory diseases and model systems. The abundance of m16.1 transcript was highly upregulated
30 (150-300-fold) in virally-induced arthritic tissues (see **Figure 32**). Dramatic increases in m16.1 expression were evident in heart or kidney organs that were transplanted to MHC mismatched recipients, *i.e.*, greater than 300-fold

induction over levels found in genetically matched transplanted tissues (see Figure 33A, 33B and 33C).

EXAMPLE 6

5 Cloning, Characterization and Functional Analysis of CATERPILLER 16.2

This example describes the characteristics of the cloned human CATERPILLER 16.2 gene. The nucleic acid and protein sequences of the cloned CATERPILLER 16.2 are set forth as SEQ ID NO:27 and SEQ ID NO:28, respectively. It has now been found that 16.2 reduces the function of
10 two crucial transcription factors involved in both inflammatory responses and cell survival, namely NF- κ B and AP-1. CATERPILLER 16.2 expression is primarily found in peripheral blood leucocytes, and is reduced by bacterial products that activate the Toll-receptor pathway, the recognition receptors for bacteria, virus, fungus and other pathogens. These results indicate that
15 CATERPILLER 16.2 is part of the Toll-receptor pathway and is involved in the regulation of immunity and cell survival.

Materials and Methods

Cloning of Full-Length CATERPILLER 16.2. The majority of the
20 CATERPILLER 16.2 gene sequence was identified as provided in EXAMPLE 1. Total RNA was prepared from the Raji cell line with TRIZOL® reagent (INVITROGEN™, Carlsbad, CA). PolyA⁺ mRNA was isolated from the total RNA using the OLIGOTEX® mRNA Mini Kit (QIAGEN®, Valencia, CA). Two gene-specific primer sets were created to clone the 5' and the 3' half of
25 CATERPILLER 16.2, separately. The overlapping pieces of the CATERPILLER 16.2 mRNA were cloned by RT-PCR using Superscript II (INVITROGEN™, Carlsbad, CA) and *Taq* polymerase (INVITROGEN™, Carlsbad, CA). The products were ligated into pCR2.1-TOPO® (INVITROGEN™, Carlsbad, CA) and sequenced for verification.

30

Plasmids. To assemble the separately cloned pieces of CATERPILLER 16.2 and to fuse the CATERPILLER 16.2 to a FLAG® epitope, overlap

extension PCR was performed with the following primer sets: 5'-
CCGGGTACCATGGACTACAAAGACGATGACGATAAAGGTGGCAGGTGG
GG GCACCAT-3' (SEQ ID NO:135) and 5'-
ATCTTCTGAATGCGACAGTCCTTC-3' (SEQ ID NO:Y); 5'-
5 AAGGACTGTCGCATTCAGAAGATC-3' (SEQ ID NO:136) and 5'-
ATAGGATCCCCAGGATCACATTTCAACAGTG-3' (SEQ ID NO:137). The
resulting product was digested with *Xho*I and *Bam*HI and cloned into a
similarly cut pcDNA3.1(-) vector (INVITROGEN™, Carlsbad, CA) using
standard methodologies.

10 *Luciferase Reporter Gene Assays.* HEK293T cells were plated at 1×10^4 cells/well in 96-well plates and transfected the following day using
FUGENE® transfection reagent (Roche, Indianapolis, IN) following the
manufacturer's recommended protocol. For NF- κ B or AP-1 reporter assays,
15 cells were transfected with 50 ng of NF- κ B-luc or AP-1-luc reporter and
various amounts of the relevant expression plasmids as indicated, maintaining
the total amount of DNA constant using pcDNA3 empty vector. The p53
reporter assays were performed in the 50 ng of p53-luc reporter plasmid, 200
ng of p53 expression vector and 400 ng of CATERPILLER 16-2 expression
20 plasmid. 20 ng/ml of TNF α or 5 ng/ml PMA was added to the indicated wells
10 hours post transfection. Cells were harvested 36 hours after transfection
and assayed for luciferase activity following standard procedures with the
equal amounts of protein as determined by the Bradford protein assay (BIO-
RAD®, Hercules, CA).

25 *RNA Preparation and Real-Time PCR.* Total RNA was isolated
according using the SV40 Total RNA System (PROMEGA™, Madison, WI)
with an additional DNase I digestion step. Real-Time PCR was performed
with the TAQMAN® sequence detection system (Applied Biosystems, Foster
30 City, CA). Primers and probes for human CATERPILLER 16.2 were: forward
5'-CTGGGAAGGGCAGTCAAG-3' (SEQ ID NO:138); reverse 5'-
TGCCTCTGTATCCTTGAGTC-3' (SEQ ID NO:139) and probe 5'-
CCCGCAGGCCCTGGATAGGACACC-3' (SEQ ID NO:140). Primers and

probes for mouse CATERPILLER 16.2 were: forward 5'-TGCTACAAGTCCGGGACAAA-3' (SEQ ID NO:141); reverse 5'-GCCCAGTTCTGGGTCATTT-3' (SEQ ID NO:142); and probe 5'-CAGCAGAGCCTCAGAGTGCTTCG-3' (SEQ ID NO:143). Primers and probes for 18S were: forward 5'-GCTGCTGGCACCAGACTT-3' (SEQ ID NO:99); reverse 5'-CGGCTACCACATCCAAGG-3' (SEQ ID NO:100); and probe 5'-CAAATTACCCACTCCCGACCCG-3' (SEQ ID NO:101). Primer-probe sets for 18S ribosomal RNA were used as internal controls. Results were normalized to the internal control and were expressed in normalized numbers.

Small Interference RNA (siRNA) Construction and Transfection.

CATERPILLER 16.2 siRNAs were generated and are useful for stably transfecting Raji and Thp-1 cells and discerning the function of CATERPILLER 16.2. The target sequence was: 5'-GGAGATCCCGGTGGACCAC-3' (SEQ ID NO:144) and the mutant sequence was: 5'-GGAGATCCtGGTGGACCAC-3' (SEQ ID NO:145).

Experimental Results

Full-Length Coding Sequence of CATERPILLER 16.2. The protein coding region of CATERPILLER 16.2 was assembled by searching human genomic sequence for CATERPILLER family genes (see EXAMPLE 1). The predicted nucleic acid and protein sequences are set forth as SEQ ID NO:25 (**Figure 34A-B**) and SEQ ID NO:26 (**Figure 34C**), respectively. The cloned sequence of CATERPILLER 16.2 was identical to the predicted CATERPILLER 16.2 sequence from nucleotides 286-2217. Nucleotide 286 of the cloned sequence corresponded to the initiation methionine in the predicted sequence. The 3' end of the cloned sequence, nucleotides 2218-3489, differed completely from the predicted sequence. The CATERPILLER 16.2 genomic sequence (NCBI) and the cloned sequence were compared to correct for any errors introduced by the cloning process. The CATERPILLER 16.2 nucleotide sequence (SEQ ID NO:27; **Figure 34D-E**) included the first ATG after an upstream, in-frame stop codon, the 3198 nucleotide ORF, and

translation stop codon. As expected, CATERPILLER 16.2 protein (SEQ ID NO:28; **Figure 34F**) contains a nucleotide binding domain (NBD) followed by a number of leucine rich repeats. Additionally, the intron-exon organization conforms to CATERPILLAR gene family; the NBD is encoded by one large
5 exon and each leucine rich repeat is encoded by individual exons of approximately 76 or 174 nucleotides. Unlike many of the CATERPILLER genes, the N-terminus of CATERPILLER 16.2 does not contain a distinguishable Pyrin or CARD domain.

10 *Expression of Human CATERPILLER 16.2 is Predominant in Immune Cells.* Real-Time PCR showed expression in Raji (B cell line), Thp-1 and U937 (myeloid monocytic cell lines) but not in the non-hematopoietic cell lines examined (**Figure 35A**). High levels of CATERPILLER 16.2 were detected in total PBMCs, with lower expression observed in adherent PBMCs relative to
15 the non-adherent population. CATERPILLER 16.2 expression was detected at lower levels in HL-60 cells and was down-regulated by 48 hours after stimulation with PMA. Thp-1 and U937 were included to indicate the relative expression of CATERPILLER 16.2. CATERPILLER 16.2 expression was not observed in the Jurkat T cell line (**Figure 35B**).

20 *Expression of Mouse 16.2 is Predominant in Immune Cells.* Real-Time PCR showed expression of mouse CATERPILLER 16.2 in 18.81 (B cell line), EL4 (T cell line) but not in the non-hematopoietic cell lines examined (**Figure 36A**). Unlike human CATERPILLER 16.2, which was found in myeloid cell
25 lines, mouse CATERPILLER 16.2 was not detected in any of the myeloid cell lines examined. Low levels of CATERPILLER 16.2 were detected in primary mouse bone marrow. High levels of mouse CATERPILLER 16.2 were detected in the spleen, thymus and peripheral blood, with a lower level observed in the lung (**Figure 36B**). Analysis of CATERPILLER 16.2
30 expression in Brewers Thioglycolate mouse peritoneal macrophages after treatment with LPS showed that CATERPILLER 16.2 was initially down-regulated at 1 hour after LPS exposure but then returned to near normal levels (**Figure 36C**).

Regulation of NF- κ B and AP-1 Activity by CATERPILLER 16.2.

HEK293T cells transiently transfected with a CATERPILLER 16.2-encoding plasmid together with NF- κ B-dependent or AP-1-dependent luciferase
5 reporters were used to examine the regulation of NF- κ B or AP-1 induction by CATERPILLER 16.2. Under the conditions indicated, CATERPILLER 16.2 appeared to inhibit NF- κ B induction by TNF α or p65 (**Figure 37A**). CATERPILLER 16.2 overexpression also markedly reduced AP-1 activity induced by PMA (**Figure 37B**). These effects were specific in that the activity
10 of other transcription factors such as p53 were not suppressed (**Figure 37A**).

EXAMPLE 7

Functional Analysis of CIAS1 and Subdomains Thereof

This example describes the characteristics of the full-length CIAS1
15 protein and two shorter, naturally occurring isoforms. It has now been found that full-length CIAS1 protein and the two shorter isoforms dramatically inhibit TNF α -induced activation of NF- κ B reporter activity. Transcriptional activity of exogenous NF- κ B p65 is also blocked by CIAS1. A truncated product, containing the nucleotide-binding and leucine-rich repeat regions but not the
20 pyrin domain of CIAS1, is responsible for this inhibition. CIAS1 suppressed TNF α -induced nuclear translocation of endogenous p65. The results provided herein indicate that CIAS1 may act as a key negative regulator of inflammation, induced to dampen NF- κ B-dependent pro-inflammatory and
25 pro-survival signals.

Materials and Methods

Cloning of CIAS1 Isoforms. A number of alternatively spliced isoforms of CIAS1 were identified during the cloning of CIAS1 from human blood cells. All CIAS1 isoforms were cloned in two pieces. Briefly, RT-PCR on total RNA
30 from PBMC was performed with gene-specific primers to amplify the 5' end (pyrin domain up to and including the NBD) and the 3' end (NBD to the last predicted LRR exon), individually. The finished full-length product was

subcloned by overlapping extension, and was 3104 bp in total long. The predicted full-length sequence corresponds to accession number NM_004895 (incorporated by reference herein in its entirety). A mouse homolog of CIAS1 was also identified and corresponds to accession number NM_145827 (incorporated by reference herein in its entirety). During the cloning of the 3' end of the human gene, several PCR products were generated, cloned, and sequenced. These products corresponded to naturally-occurring splice variants of CIAS1, missing one or more LRR-containing exons. One such isoform was the originally identified Cryopyrin, with exons 4 and 6 deleted in-frame (accession number AY092033; incorporated by reference herein in its entirety). Also identified, cloned, sequenced, and analyzed was a previously undescribed isoform with exon 4 deleted in-frame. This isoform was designated 'FgCIAS1 Del4' with nucleotide and deduced protein sequences set forth as SEQ ID NO:148 (**Figure 42K-L**) and SEQ ID NO:149 (**Figure 42M**), respectively.

Monocyte Preparation and Real-Time PCR Analysis. Primary human monocytes were isolated from normal donor buffy coat preparations (American Red Cross, Durham, NC). PBMCs were obtained using a standard FICOLL®-centrifugation procedure. The non-adherent fraction was removed and fresh medium was subsequently added alone or with stimulant as indicated. LPS from *E.coli* (026:B6, Sigma Chemicals, St. Louis, MO) or *S. enteritidis* (Sigma, St. Louis, MO) was added to 200 ng/ml; LTA from *S. aureus* (Sigma, St. Louis, MO) or PGN from *S. aureus* (Fluka Biochemika, Buchs, Switzerland) to 1 µg/ml; Poly I:C (Amersham, Piscataway, NJ) to 10 µg/ml; CpG oligonucleotide (ODN 1668) or control GpC oligonucleotide (ODN 1720, both from TIB MolBiol, Berlin, Germany) to 1 µM. Cells were stimulated for 1 hour at 37°C, except as indicated. In select experiments, freshly isolated monocytes were cultured with signaling pathway inhibitors, DMSO control or medium alone for 20 minutes prior to LPS stimulation. The MAPK inhibitor U0126 (PROMEGA™, Madison, WI) and the p38 inhibitor SB203580 (CALBIOCHEM®, San Diego, CA) were used at 10 µM final concentration, the PI3K inhibitor Wortmannin at 100 nM. These concentrations have been

shown to be effective in inhibiting their intended target (MacKeigan, *et al.* (2000) *J. Biol. Chem.* 275:38953; Yao and Cooper (1995) *Science* 267:2003). RNA was isolated according to the manufacturer's instructions (SV Total RNA Isolation, PROMEGA™, Madison, WI) and first strand synthesis was

5 performed using standard methods (MMLV-RT).

Real-Time PCR analyses were performed on the ABI Prism 7700 instrument (ABI, PerkinElmer, Foster City, CA) (Wong, *et al.* (2002) *J. Immunol.* 169:3112). CIAS1 gene expression was determined using the following intron-spanning primers for amplification: forward primer: 5'-

10 GGCATATCACAGTGGGATTC-3' (SEQ ID NO:146) and reverse primer: 5'-GATCTTCGCTGCGATCAAC-3' (SEQ ID NO:147). Amplification of 18S RNA was performed using a standard method (Wong, *et al.* (2002) *J. Immunol.* 169:3112). CIAS1 expression was quantitated by comparing values obtained to a standard curve generated with plasmid DNA. All CIAS1 values obtained

15 were normalized to 18s RNA (CIAS1 molecules / Attomole of 18S) and reported as differences in fold induction of CIAS1 over levels of CIAS1 in untreated, resting monocyte cultures.

Cell Transfection and Luciferase Assays. HeLa cells (American Type

20 Tissue Collection, Manassas, VA) were transfected with the indicated quantities of the following FLAG®-tagged CIAS1 constructs: full-length wild-type (Fg CIAS1), CIAS1 Deletion exon 4 (Fg Del4), CIAS1 Deletion exon 4 and 6 (Fg Del4 Del6), CIAS1 truncation mutants (CIAS1 Pyrin, CIAS1 Pyrin/NBD, CIAS1 NBD/LRR, and CIAS1 LRR), or pcDNA3 together with 100

25 ng of 3x-NF-κB-Luciferase using FUGENE6™ (Roche, Indianapolis, IN). 24 hours post-transfection, cells were stimulated with TNFα (10 ng/ml) or transfected with either empty vector or pCMV4T-p65 (500 ng/well) and incubated at 37°C for an additional 24 hours. Cells were then lysed and luciferase quantitated using standard methodologies. The p53-luciferase

30 control reporter construct was used at 500 ng/well.

Immunofluorescent Staining and Quantitation. HeLa cells were transfected alone or in combination with 500 ng/well DR-luciferase, 1.5

µg/well FLAG®-CIAS1, or 1.5 µg/well pcDNA3 using FUGENE™. 24 hours after transfection, cells were stimulated with TNFα (10 ng/ml), CIITA (100 ng/well, pcDNA3 (100 ng/well) or medium alone for 30 min at 37°C as indicated. Twenty-four hours post-stimulation the wells were washed 3x PBS, lysed for 15 minutes at room temperature and Luciferin substrate quantitated as per standard protocol. Other staining was performed using well-known methods. Endogenous p65 was visualized using a rabbit anti-p65 Ab (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and goat anti-rabbit-biotin/Avidin-Texas Red secondary Abs (Vector Laboratories, Burlingame, CA). FLAG®-tagged CIAS1 was visualized using the FLAG® antibody M5 (Sigma, St. Louis, MO) and goat anti-mouse FITC (BD Pharmingen, San Diego, CA). Nuclei were counterstained using DAPI (Vector Laboratories, Burlingame, CA). The subcellular localization of p65 was assessed in 100 CIAS1^{low/neg} cells and compared to p65 in 100 CIAS1⁺ cells in three double-blind studies.

Experimental Results

CIAS1 is Induced by Stimulants of Toll-Like Receptor Signaling.

Expression of CIAS1 in peripheral blood cells was determined to be primarily restricted to monocytes (Manji, *et al.* (2002) *J. Biol. Chem.* 277:11570). Real-time PCR analysis was conducted to quantitate levels of CIAS1 RNA in both resting and activated cells to examine the inducibility of CIAS1 in primary human monocytes. LTA, PGN, poly I:C, LPS, and CpG oligonucleotides are well characterized stimulators of TLR-2, -3, -4, and -9, (Schwandner, *et al.* (1999) *J. Biol. Chem.* 274:17406; Alexopoulou, *et al.* (2001) *Nature* 413:732; Poltorak, *et al.* (1998) *Science* 282:2085; Hemmi, *et al.* (2000) *Nature* 408:740). Administration of LTA, LPS, PGN, or Poly I:C to primary human monocytes elicited a robust induction of CIAS1 expression (**Figure 38A**). No changes in CIAS1 gene expression were observed with CpG oligonucleotides although this may be due to the restricted expression of TLR9 on human plasmacytoid dendritic cells and B cells whose presence in the preparation was not ascertained.

E. coli LPS acted rapidly to induce an approximate 15-fold increase in CIAS1 expression within 30 minutes of stimulation, with expression reaching

over 20-fold by 1 hour (**Figure 38B**). This induction was reproducible within experiments and between multiple donor blood preparations. The expression of CIAS1 may be under tight regulation as CIAS1 RNA was low in resting monocytes, induced strongly within an hour of LPS stimulation, and returning to baseline levels within 12 hours following LPS addition.

LPS Induction of CIAS1 Via the MAPK/p38 Pathways. Primary human monocytes were treated with MAPK, p38, or PI3K inhibitors prior to LPS stimulation to determine the pathways involved in CIAS1 expression.

10 Treatment with the MAPK pathway inhibitor U0126 or the p38 pathway inhibitor SB203580 led to a reduction in LPS-induced CIAS1 RNA while the PI3K pathway inhibitor Wortmannin had no effect (**Figure 38C**). Thus, LPS induction utilizes the MAPK / p38 but not PI3K signaling pathways to induce CIAS1 expression.

15

Multiple Isoforms of CIAS1 Inhibit NF- κ B Reporter Activity. The rapid induction of CIAS1 by immunostimulatory molecules indicated that CIAS1 plays a role in mediating the inflammatory response. As NF- κ B activity has been intimately linked to inflammation (Li and Verma (2002) *Nature Rev.*

20 *Immunol.* 2:725), NF- κ B activity in the presence of transfected CIAS1 was examined. Transfection of full-length CIAS1 or either of two shorter, naturally-occurring splice variants of CIAS1 (**Figure 39A**) did not lead to activation of NF- κ B-Luciferase. Unexpectedly, relative decreases in basal NF- κ B-luciferase activity in the CIAS1 positive lanes was observed. TNF α induces NF- κ B, and
25 in the experiments conducted herein, TNF α also elicited NF- κ B reporter activity (**Figure 39B**, first two lanes). Since the observed decreases in basal activity suggested a possible inhibitory role for CIAS1, we tested the ability of CIAS1 to inhibit TNF α -induced NF- κ B-Luciferase. As shown in **Figure 39B**, overexpression of all three CIAS1 isoforms led to a strong, dose-dependent
30 inhibition of TNF α -induced NF- κ B-luciferase.

Many signaling pathways leading to the activation of NF- κ B share a common mechanism of action that liberates the p50 and p65 subunits of NF-

κ B from the I κ B complex, allowing them to be phosphorylated and imported into the nucleus. Thus, the effects of CIAS1 on p65-induction of the NF- κ B-luciferase construct were analyzed to determine the position CIAS1 occupies in the NF- κ B pathway. CIAS1 dramatically inhibited the ability of p65 to
5 activate the NF- κ B-luciferase reporter in a dose-dependent fashion (**Figure 39C**). This indicates that CIAS1 functions at the distal end of NF- κ B signaling by affecting p65 function. p53 induction of a p53-responsive luciferase construct was largely unaffected reflecting the specificity of CIAS1. Additionally, an HA-tagged CIAS1 showed identical results.

10

CIAS1 Functions in the Cytoplasm. Indirect immunofluorescence studies were performed to visualize the subcellular localization of overexpressed CIAS1. Full-length CIAS1 localizes to the cytoplasm in the absence of any stimulus (Manji, *et al.* (2002) *J. Biol. Chem.* 277:11570), and
15 the effects of cellular stimulation on the localization of CIAS1 were assessed by transiently transfecting HeLa cells with the concentration of CIAS1 shown to inhibit NF- κ B-luciferase and visualizing FLAG®-tagged CIAS1. Twenty minutes of TNF α stimulation potently induced endogenous p65 to enter the nucleus but did not lead to nuclear translocation of FgCIAS1. These results
20 indicate that CIAS1 functions in the cytoplasm to inhibit NF- κ B.

CIAS1 Inhibits Nuclear Translocation of p65. Inhibition of NF- κ B may occur at any of several stages in the activation cascade. The observation that CIAS1 inhibits exogenously transfected 'free' p65 indicated that one function
25 of CIAS1 may be to inhibit nuclear translocation of the p65 subunit. Thus, HeLa cells were transiently transfected with CIAS1 and TNF α -induced nuclear translocation of endogenous p65 was analyzed. TNF α stimulation of NF- κ B lead to rapid movement of p65 into the nuclear compartment (Beg, *et al.* (1993) *Mol. Cell Biol.* 13:3301). In the presence of CIAS1, a significant
30 reduction in the amount of nuclear p65 was observed in response to TNF α . A double-blind numerical analysis of this effect was performed (**Figure 40**).

Inhibition of TNF α Signaling is Mediated by the Nucleotide-Binding and Leucine-Rich Repeat Regions of CIAS1. A series of FLAG®-tagged deletion constructs of CIAS1 were generated (**Figure 41A**) and tested to determine the inhibitory nature of CIAS1 in TNF α signaling. The pyrin construct
5 contained nucleic acid sequences encoding the pyrin domain, herein set forth as SEQ ID NO:29 (**Figure 42A**) and SEQ ID NO:30 (**Figure 42B**), respectively. The pyrin/NBD construct contained nucleic acid sequences encoding the pyrin and NBD domains, herein set forth as SEQ ID NO:31 (**Figure 42C-D**) and SEQ ID NO:32 (**Figure 42E**), respectively. The
10 NBD/LRR construct contained nucleic acid sequences encoding the NBD/LRR domains, herein set forth as SEQ ID NO:33 (**Figure 42F-G**) and SEQ ID NO:34 (**Figure 42H**), respectively. The LRR construct contained nucleic acid sequences encoding the LRR domain, herein set forth as SEQ ID NO:35 (**Figure 42I**) and SEQ ID NO:36 (**Figure 42J**), respectively. The NBD
15 and LRR regions together inhibited TNF α -induced NF- κ B activity as did the full-length construct (**Figure 41B**). Deletion of the amino-terminal Pyrin domain had no deleterious effect on inhibition and transfection of the Pyrin domain alone served to activate NF- κ B- luciferase above and beyond TNF α stimulation.

20
Regulation of CIITA Activity. The observation that CIAS-1 regulates NF- κ B activity indicates that CIAS-1 may play a role in either inflammation or apoptosis, or both. CIAS-1 may be an inflammatory mediator which regulates other molecules involved in the inflammatory process. One arm of the
25 inflammatory response involves antigen presentation through the MHC class I and/or MHC class II pathways. As CIITA is widely regarded as the master regulator of MHC class II gene transcription and to a lesser extent affects MHC class I transcription, the effect of CIAS-1 on CIITA activity was analyzed. Using an MHC class II reporter construct, DR-Luciferase, it was found that
30 CIAS-1 inhibits the ability of overexpressed CIITA to activate DR-Luciferase in HeLa cells (**Figure 43**).

The combination of ASC and CIAS1 causes the induction of NF- κ B, but ASC alone has the opposite effect by inhibiting NF- κ B activation (Stehlik, *et al.* (2002) *J. Exp. Med.* 196:1605). Similarly, the results provided herein revealed that CIAS1 alone, reduced TNF α and NF- κ B responses. Together, these results indicate that the balance of ASC and CIAS1 critically determine the extent of inflammatory responses, and that alone, either may serve as an important suppressor molecule. Notably, NF- κ B nuclear translocation was routinely detectable within 10-30 minutes after cell activation while increases in CIAS1 RNA were observed 30-60 minutes after stimulation. Thus, CIAS1 may be induced to limit the extent of the pro-inflammatory cytokine cascade, preventing hyper-inflammation seen in autoinflammatory syndrome patients.

The invention is described by the following claims, with equivalents of the claims to be included therein.

That which is claimed is:

1. An isolated nucleic acid encoding a polypeptide selected from the group consisting of:

- 5 (a) a Monarch-1 polypeptide;
 (b) a CATERPILLER 11.2 polypeptide;
 (c) a CATERPILLER 11.3 polypeptide;
 (d) a CATERPILLER 16.1 polypeptide;
 (e) a CATERPILLER 16.2 polypeptide;
10 (f) a CIAS1 polypeptide comprising the amino acids encoded by
 exon 6 and lacking the amino acids encoded by exon 4; and
 (g) a functional fragment of any of (a) to (f).

2. An isolated nucleic acid encoding a functional fragment of a
15 CIAS1 polypeptide.

3. The isolated nucleic acid of Claim 1 or Claim 2, wherein said polypeptide comprises a nucleotide-binding domain and a leucine rich repeat.

20 4. The isolated nucleic acid of any of Claims 1-3, wherein said isolated nucleic acid comprises a nucleotide sequence selected from the group consisting of:

- (a) the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ
 ID NO:5, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:17, SEQ ID
25 NO:19, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:33 or SEQ
 ID NO:148;
 (b) a nucleotide sequence consisting essentially of a fragment of
 the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ
 ID NO:5, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:17, SEQ ID
30 NO:19, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:33 or SEQ
 ID NO:148, wherein said fragment encodes a functional
 polypeptide;

- (c) a nucleotide sequence that hybridizes to the complement of the nucleotide sequences of (a) or (b) under stringent hybridization conditions and encodes a functional polypeptide;
- (d) a nucleotide sequence that differs from the nucleotide sequences of (a), (b) and (c) above due to the degeneracy of the genetic code.

5 5. The isolated nucleic acid of any of Claims 1, 3 or 4 encoding the polypeptide of SEQ ID NO:2 or a functional fragment thereof.

10 6. The isolated nucleic acid of any of Claims 1, 3 or 4 encoding the polypeptide of SEQ ID NO:4 or a functional fragment thereof.

15 7. The isolated nucleic acid of any of Claims 1, 3 or 4 encoding the polypeptide of SEQ ID NO:6 or a functional fragment thereof.

 8. The isolated nucleic acid of any of Claims 1, 3 or 4 encoding the polypeptide of SEQ ID NO:8 or a functional fragment thereof.

20 9. The isolated nucleic acid of any of Claims 1, 3 or 4 encoding the polypeptide of SEQ ID NO:14 or a functional fragment thereof.

 10. The isolated nucleic acid of any of Claims 1, 3 or 4 encoding the polypeptide of SEQ ID NO:18 or a functional fragment thereof.

25 11. The isolated nucleic acid of any of Claims 1, 3 or 4 encoding the polypeptide of SEQ ID NO:20 or a functional fragment thereof.

30 12. The isolated nucleic acid of any of Claims 1, 3 or 4 encoding the polypeptide of SEQ ID NO:24 or a functional fragment thereof.

 13. The isolated nucleic acid of any of Claims 1, 3 or 4 encoding the polypeptide of SEQ ID NO:28 or a functional fragment thereof.

14. The isolated nucleic acid of any of Claims 2- 4 encoding the polypeptide of SEQ ID NO:34 or a functional fragment thereof.

5 15. The isolated nucleic acid of any of Claims 2- 4 encoding the polypeptide of SEQ ID NO:149 or a functional fragment thereof.

16. The isolated nucleic acid of any of Claims 1-4, wherein said isolated nucleic acid comprises a nucleotide sequence that encodes a
10 functional polypeptide that has at least about 80% amino acid sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:34, SEQ ID NO:149 and a functional fragment of any of the foregoing.

15

17. An isolated polypeptide encoded by the isolated nucleic acid of any of Claims 1-16.

18. The isolated polypeptide of Claim 17, wherein said isolated
20 polypeptide consists essentially of an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:34 and SEQ ID NO:149.

25 19. The isolated polypeptide of Claim 17, wherein said isolated polypeptide consists essentially of a functional fragment of a polypeptide having an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:24, SEQ
30 ID NO:28, SEQ ID NO:34, and SEQ ID NO:149.

20. A cell comprising the isolated nucleic acid of any of Claims 1-16.

21. A cell comprising the isolated polypeptide of any of Claims 17-19.

22. A fusion protein comprising the polypeptide of any of Claims 17-19.

23. An isolated nucleic acid encoding the fusion protein of Claim 22.

24. An antibody that specifically binds to the polypeptide of any of Claims 16-18.

25. The antibody of Claim 24, wherein the antibody is a monoclonal antibody.

26. The antibody of Claim 24 or Claim 25, wherein the antibody is attached to a detectable moiety.

27. A method of modulating the cellular activity of a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2, and a functional fragment thereof, comprising introducing into a cell a compound that modulates the activity of the polypeptide in an amount effective to modulate the activity of the polypeptide in the cell.

28. The method of Claim 27, wherein the compound is an isolated nucleic acid encoding the polypeptide.

29. The method of Claim 27, wherein the compound is selected from the group consisting of an antisense oligonucleotide and a siRNA that targets the nucleic acid encoding the polypeptide.

30. The method of Claim 27, wherein the compound is an antibody that binds to the polypeptide.

31. A method of modulating cellular inflammatory responses,
5 comprising introducing into a cell a compound that modulates the activity of a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2, and a functional fragment thereof, said compound introduced in an amount effective to modulate cellular inflammatory
10 responses.

32. The method of Claim 31, wherein the compound is an isolated nucleic acid encoding the polypeptide.

15 33. The method of Claim 31, wherein the compound is selected from the group consisting of an antisense oligonucleotide and a siRNA that targets the nucleic acid encoding the polypeptide.

34. The method of Claim 31, wherein the compound is an antibody
20 that binds to the polypeptide.

35. A method of modulating apoptosis, comprising introducing into a cell a compound that modulates the activity of a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER
25 11.3, CATERPILLER 16.1, CATERPILLER 16.2, and a functional fragment thereof, said compound introduced in an amount effective to modulate apoptosis.

36. The method of Claim 35, wherein the compound is an isolated
30 nucleic acid encoding the polypeptide.

37. The method of Claim 35, wherein the compound is selected from the group consisting of an antisense oligonucleotide and a siRNA that targets the nucleic acid encoding the polypeptide.

5 38. The method of Claim 35, wherein the compound is an antibody that binds to the polypeptide.

39. A method of modulating Toll-like receptor activity, comprising introducing into a cell a compound that modulates the activity of a polypeptide
10 selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2, and a functional fragment thereof, said compound introduced in an amount effective to modulate Toll-like receptor activity.

15 40. The method of Claim 39, wherein the compound is an isolated nucleic acid encoding the polypeptide.

41. The method of Claim 39, wherein the compound is selected from the group consisting of an antisense oligonucleotide and a siRNA that
20 targets the nucleic acid encoding the polypeptide.

42. The method of Claim 39, wherein the compound is an antibody that binds to the polypeptide.

25 43. The method according to any of Claims 27-42, wherein the cell is a cultured cell.

44. The method according to any of Claims 27-42, wherein the cell is a cell *in vivo*.

30

45. A method of identifying a compound that binds to a polypeptide selected from the group consisting of Monarch-1, CATERPILLER 11.2,

CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2, and a functional fragment of any of the foregoing, comprising:

contacting the polypeptide with a test compound under conditions whereby binding between the polypeptide and the test compound can be
5 detected; and

detecting binding between the polypeptide and the test compound.

46. A method of identifying a compound that modulates the activity of a polypeptide selected from the group consisting of Monarch-1,
10 CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2, and a functional fragment of any of the foregoing, comprising:

contacting the polypeptide with a test compound under conditions whereby modulation of the activity of the polypeptide can be detected; and
15 detecting modulation of the activity of the polypeptide.

47. A method of identifying a compound that can modulate inflammatory responses, comprising:

contacting a polypeptide selected from the group consisting of
20 Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2 and a functional fragment of any of the foregoing with a test compound under conditions whereby modulation of the activity of the polypeptide can be detected; and

detecting modulation of the activity of the polypeptide, thereby
25 identifying a compound that can modulate inflammatory responses.

48. A method of identifying a compound that can modulate apoptosis, comprising:

contacting a polypeptide selected from the group consisting of
30 Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2 and a functional fragment of any of the foregoing with a test compound under conditions whereby modulation of the activity of the polypeptide can be detected; and

detecting modulation of the activity of the polypeptide, thereby identifying a compound that can modulate apoptosis.

49. A method of identifying a compound that can modulate the Toll-like receptor pathway, comprising:

contacting a polypeptide selected from the group consisting of Monarch-1, CIAS1, CATERPILLER 11.2, CATERPILLER 11.3, CATERPILLER 16.1, CATERPILLER 16.2 and a functional fragment of any of the foregoing with a test compound under conditions whereby modulation of the activity of the polypeptide can be detected; and

detecting modulation of the activity of the polypeptide, thereby identifying a compound that can modulate the Toll-like receptor pathway.

50. The method of any of Claims 45-49, wherein the method is carried out in a cell comprising the polypeptide.

51. The method of Claim 50, wherein the cell comprises an isolated nucleic acid comprising a nucleotide sequence encoding the polypeptide.

52. The method of Claim 51, wherein the cell is stably transformed with the isolated nucleic acid.

53. The method of any of Claims 45-49, wherein the method is carried out as a cell-free assay.

54. The method of any of Claims 45-49, wherein the method is carried out in a transgenic non-human mammal comprising an isolated nucleic acid comprising a nucleotide sequence encoding the polypeptide.